

# Agilent 2100 Bioanalyzer Troubleshooting and Maintenance Guide for Molecular Assays

Edition March 01



Agilent Technologies



## WARNING

For details of safety, see the *Site Preparation and Safety Manual* for the Agilent 2100 Bioanalyzer.

The Agilent 2100 Bioanalyzer is marked with this symbol when the user should refer to the *Site Preparation and Safety Manual* in order to protect the Agilent 2100 Bioanalyzer against damage.



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# Welcome

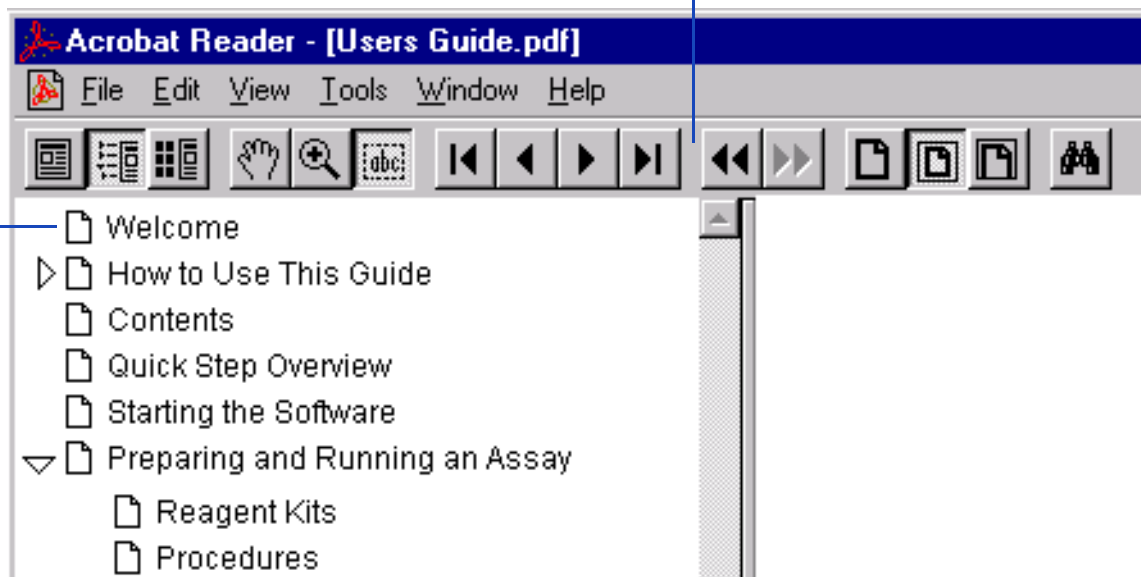
Welcome to the Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide. This online manual provides novice and advanced users with information needed to successfully run assays with the Agilent 2100 Bioanalyzer.

A quick look at **How to Use This Guide** on page 4 explains how easy it is to use this online manual and helps you to get started.

# How to Use This Guide

Use the interactive bookmarks in this frame to choose your desired topic.

Use Acrobat Reader's navigation bar to move around within a topic, see Navigating within Acrobat Reader



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Here's the current page number

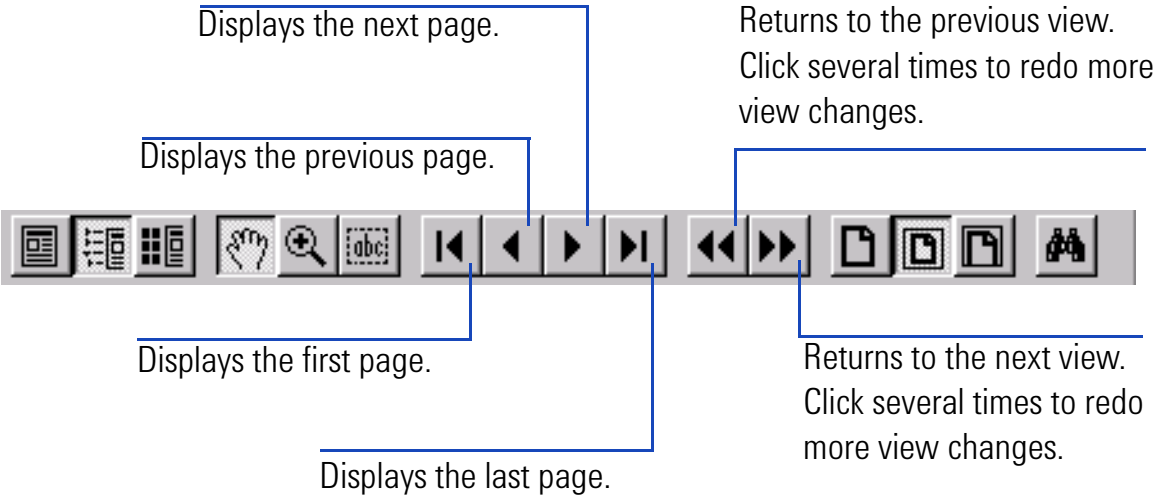
▲ displays previous page

▼ displays next page

Click here to go to the index

# Navigating within Acrobat Reader

When you've chosen a topic with the bookmarks, use the buttons in Acrobat Reader's tool bar to move around within a topic.



For more information, see the Reader Online Guide in the Help menu.

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# Essential Measurement Practices

This section lists all user relevant hints on handling tools, chips, reagents and Agilent 2100 Bioanalyzer. For the latest information on assay-related hints, go to the Lab-on-a-Chip web site at:

<http://www.agilent.com/chem/labonachip>

## Tools and Handling

- Always wear gloves when handling chips to prevent them from getting contaminated.
- When pipetting sample, use pipette tips that are small enough. Pipette tips that are too large will lead to poor quantitation accuracy.
- Change pipette tips between two pipetting steps to avoid cross-contamination.
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well leads to bubbles and poor results. Holding the pipette at a slight angle will ensure proper dispensing of the liquid.
- Use a new syringe and cleaning chip with each new LabChip Kit.

## Chip Priming Station

- For the correct position of the syringe clip and base plate please refer to the appropriate Reagent Kit Guide.
- Replace the syringe with each new LabChip Kit.
- Check the performance of the chip priming station by applying the seal test on a monthly basis. For details see **Maintaining the Chip Priming Station—271**. If necessary replace the gasket and/or adapter (reorder no. for gasket kit: G2938-68716).



## Reagents and Reagent Mixes—General

- Handle and store all reagents according to the instructions given in the specific Reagent Kit Guide.
- Keep all reagents and reagent mixes (for example, the gel-dye mixture) refrigerated at 4 °C when not in use for more than 1 hour. Reagents might decompose, leading to poor measurement results.
- Allow all reagents and samples to equilibrate to room temperature for 30 min before use.
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.

## Gel and Gel-Dye

- Use gel-dye mixture within four weeks of preparation. The gel-dye mixture might decompose and lead to poor measurement results.

## Samples

- Refer to the assay specific Reagent Kit Guides for maximum allowed sample and salt concentration.
- For RNA assays: Heat denature all RNA samples and RNA ladder for 2 min at 70°C before use.

- For protein assays: Use 0.5 ml tubes for denaturation. Using larger tubes lead to poor results.

## **Chips**

- Prepared chips must be used within 5 minutes. Reagents might evaporate, leading to poor results.
- Vortex chips at the appropriate time of 1 minute. Inappropriate vortexing leads to poor results. Use only the IKA vortexer for chip vortexing. Replace the chip adapter (p/n 5022-2190) if it is wore out.
- Do not touch wells of the chip. The chip could get contaminated and this leads to poor measurement results.
- Do not leave any wells of the chip empty, or the assay will not run properly. Add 1  $\mu$ l of sample buffer to each unused sample well so that the total liquid volume in each well is at least 6  $\mu$ l. For protein assays: pipette a sample or ladder replicate in any empty sample well.
- Do not touch the underside of the chip.

## **Agilent 2100 Bioanalyzer**

- Don't touch the Agilent 2100 Bioanalyzer during a run and never place it on vibrating ground.

- Do not use force to press the chip in the receptacle of the Agilent 2100 Bioanalyzer. The electrode assembly might get damaged when you close the lid. Check if the chip selector is in the correct position.
- Clean electrodes on a daily basis using the cleaning chip. For more details, see Maintenance—246.
- Clean electrodes on a quaterly basis using a toothbrush and distilled water. For more details, see Maintenance—246.
- Clean the focusing lens once a month (or after any liquid spill) using isopropanol see Lens Maintenance—270.

# Decontamination Procedure for RNA Assays

Perform the following decontamination/cleaning procedure on a daily basis before running any RNA assays. See **Maintenance—246** for more information regarding the use of the electrode cleaner and/or the procedures for cleaning and/or decontamination.

## Decontamination:

- 1 Slowly fill an electrode cleaner with 350  $\mu$ L RNaseZAP. (Label this electrode cleaner "for RNase ZAP.")
- 2 Open the lid, place the electrode cleaner in the instrument, and close the lid for approximately 1 minute.
- 3 Open the lid, remove the RNase ZAP electrode cleaner, and store it for future use. You can reuse this electrode cleaner for all the chips in the kit. Empty the electrode cleaner for overnight storage.
- 4 Then follow the instructions below for cleaning the electrodes.

## Cleaning:

- 1 Slowly fill another electrode cleaner with 350  $\mu$ L RNase-free water. (Label this electrode cleaner "RNase-free water.")
- 2 Open the lid, load this electrode cleaner into the instrument and close the lid, immersing the electrodes in the water.

- 3 After approximately 10 seconds, remove the electrode cleaner. Put this electrode cleaner aside for future use as well.
- 4 Wait another 10 seconds for the water on the electrodes to evaporate.

# Troubleshooting the Instrument Communication

## Communication

To check whether your PC communicates with the Agilent 2100 Bioanalyzer:

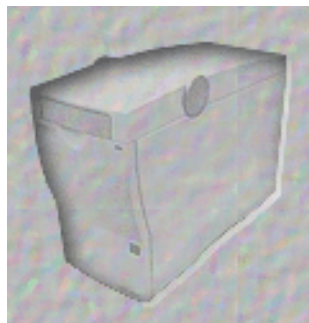
- 1 Start the Agilent 2100 Bioanalyzer software.
- 2 Open and close the lid—the icon in the main screen should change from open to closed:



Lid open



Lid closed



Instrument switched off or not connected to PC

If the icon doesn't change:

- ❑ Check whether the status indicator is on. If it is off, replace the fuses as described under Maintenance.
- ❑ Check whether the status indicator is red. If it is red, turn off line power to the Agilent 2100 Bioanalyzer and turn on again. If the problem persists, call Agilent Technologies.
- ❑ Check that the RS232 communication cable is connected correctly.
- ❑ Check if another hardware device is connected to your computer via RS232 cable
- ❑ Check the Com port settings in the Agilent 2100 Bioanalyzer software, see Changing the COM-Port Settings—16.
- ❑ Replace the RS232/Multiport cable.
- ❑ Reinstall the Agilent 2100 Bioanalyzer software.

If the Agilent 2100 Bioanalyzer will still not communicate, call Agilent Technologies.

# Changing the COM-Port Settings

**NOTE** The number of COM-Ports available depends on your bundle PC:

Laptop system: only one COM-Port is available.

Single instrument system: two COM-Ports (A and B) are available.

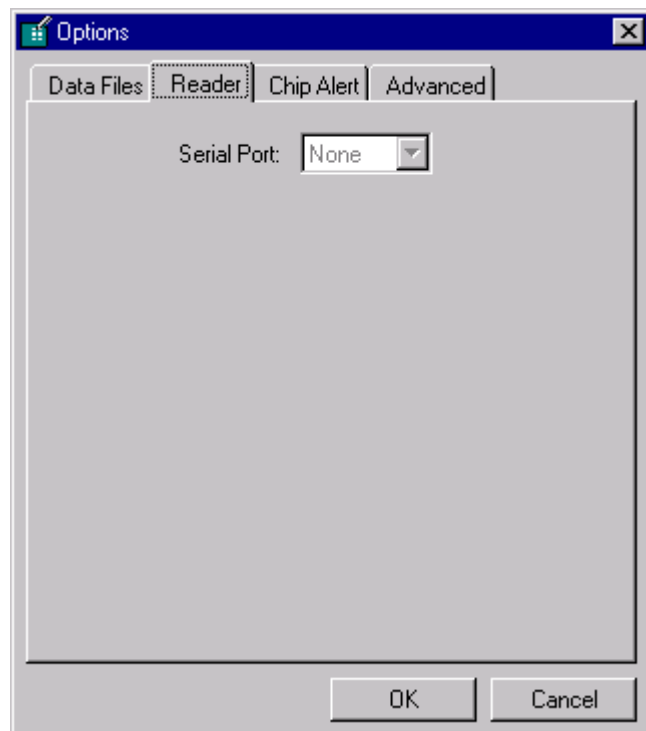
Multi-instrument system: up to 4 COM-Ports (1 to 4) are available.

In case of a Multi-instrument system a Multi Port Card (e.g. RocketPort PCI/Quad DB9) is installed in your PC. Only connect the Agilent 2100 bioanalyzer via the Multi Port Card/Multi Port cable with your computer. Do not use the default COM-Ports of your PC (Port A or B). Setting-up the Multi Port Card might be necessary after PC-repair or reinstallation of the operating system.



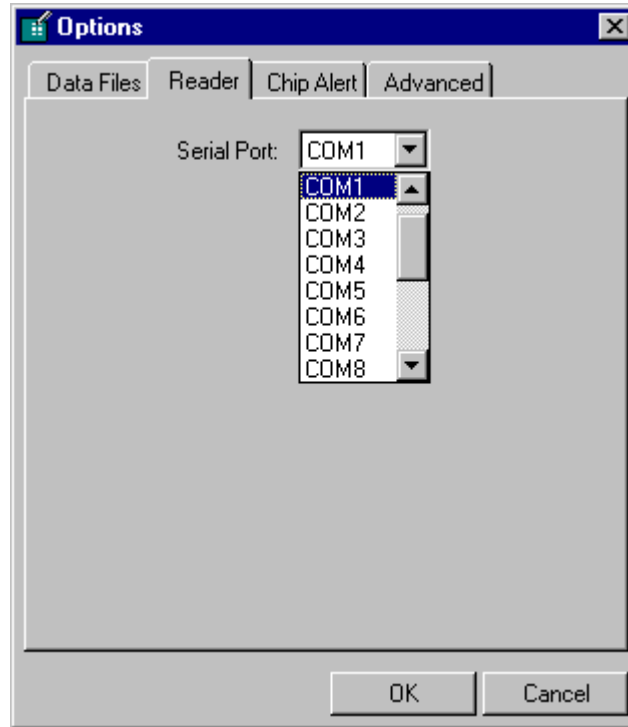
## NOTE

If you have selected a demo assay, it is not possible to change the COM-settings:



# Laptop and single instrument system

- 1 Choose Options from the Tools menu. The dialog box that appears contains four tabbed sections, labeled Data Files, Reader, Chip Alert, and Advanced. Click the Reader tab. The dialog box should look like this (your COM setting may be different):



- 2 Try choosing a COM-Port setting that is different from the one that is currently selected. If you know which port is in use on the PC, choose that port.

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**NOTE**

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If you have a laptop connected to your instrument you must choose COM1.

- 3** Check the icon of the Agilent 2100 Bioanalyzer on the screen. If it is no longer dimmed, communication between the Agilent 2100 Bioanalyzer and PC is working properly. In addition hardware information is displayed.

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**NOTE**

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If you have a PC connected to your instrument and the icon is still dimmed, repeat step 2, choosing a different Com Port each time, until it is not dimmed anymore.

If you cannot resolve the communication problem in this way, check the troubleshooting help for more information.

- 4** When you are finished with the Options dialog box, click the OK button to close it.

# Multi-instrument system

Setting up the Serial Interface of your Agilent 2100 bioanalyzer Multi-Instrument System (G2942AA)

## General

In case you have to re-install your operating system using the recovery CD provided, you also need to setup the RocketPort PCI/Quad DB9 Multi Port Serial card in your PC. This will enable multi-instrument support for the Agilent Technologies bioanalyzer 2100 again.

### NOTE

Reloading the software might be necessary after severe PC system crashes or in case part of the software installation has been corrupted. Please make sure to have the RocketPort PCI/Quad DB9 Multi Port Serial card properly installed before trying to configure the serial ports as described in this document.

The necessary steps to enable multi-instrument support for the Agilent Technologies bioanalyzer 2100 depends on the installed operating system. Choose:

MultiPort Card Setup Process (Windows 2000)

MultiPort Card Setup Process (Windows NT)

# MultiPort Card Setup Process (Windows 2000)

## NOTE

After re-installation of the operating system, make sure that the MultiPort Cable (reorder number G2938-81610) is plug- in. Windows 2000 will automatically detect the MultiPort Card and install the necessary driver.

The setup process of the MultiPort Card requires 2 steps:

Step1: Disabling the standard serial ports

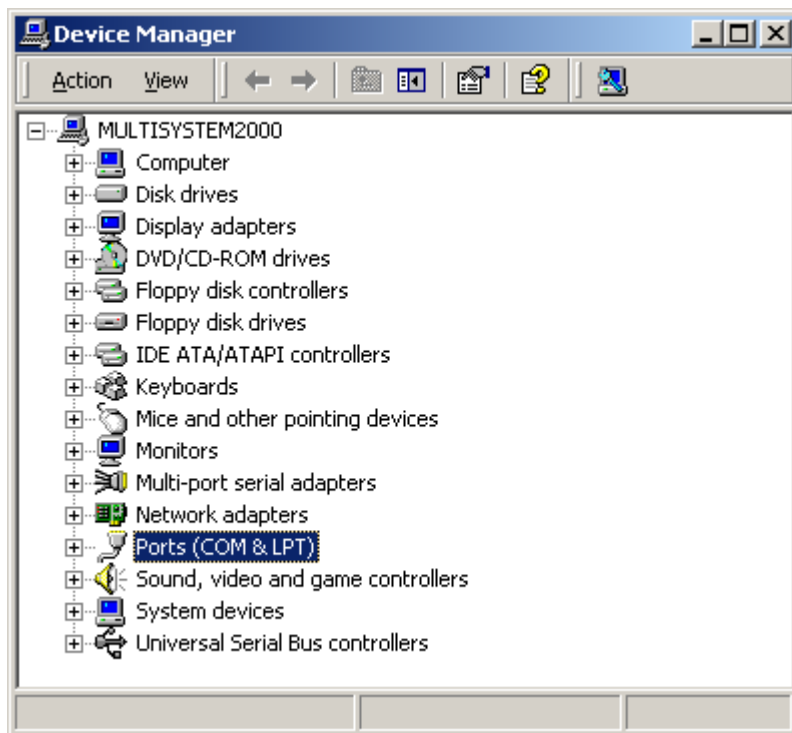
Step 2: Assigning COM1 to COM4 to the RocketPort Card

The setup process is described on the following pages. For support on configuring the RocketPort PCI/Quad DB9 Multi Port Serial card, please contact Agilent service personnel.

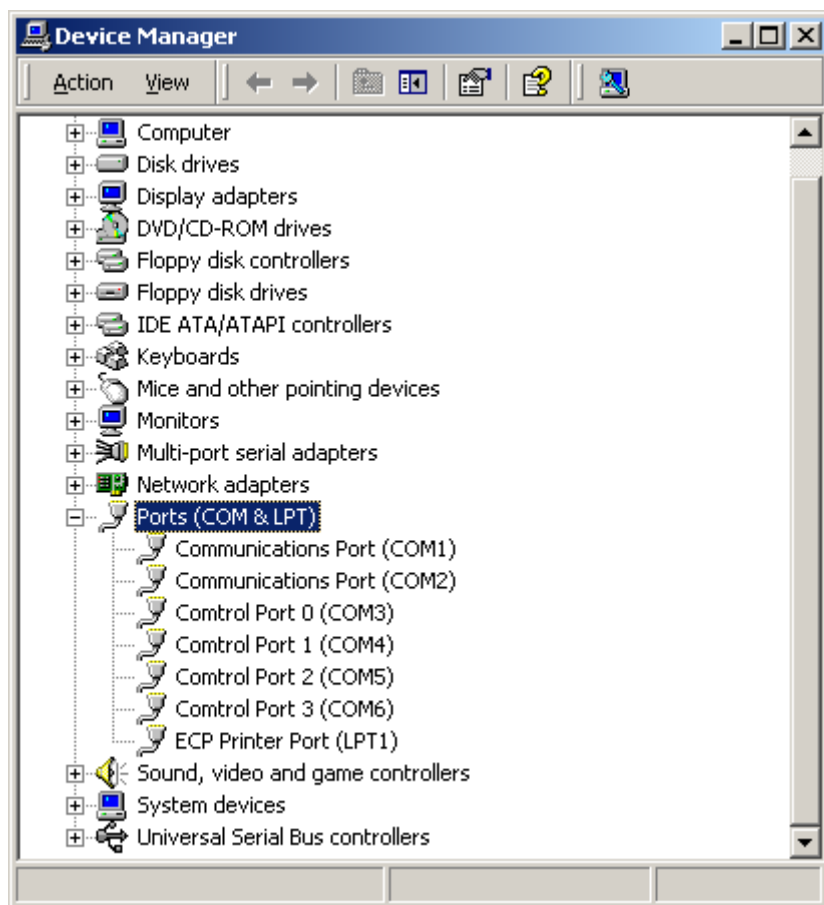
## Step1: Disabling the Standard PC Serial Ports (COM1 and COM2)

The embedded serial ports of your PC must be disabled before you can use your RocketPort card. To do so,

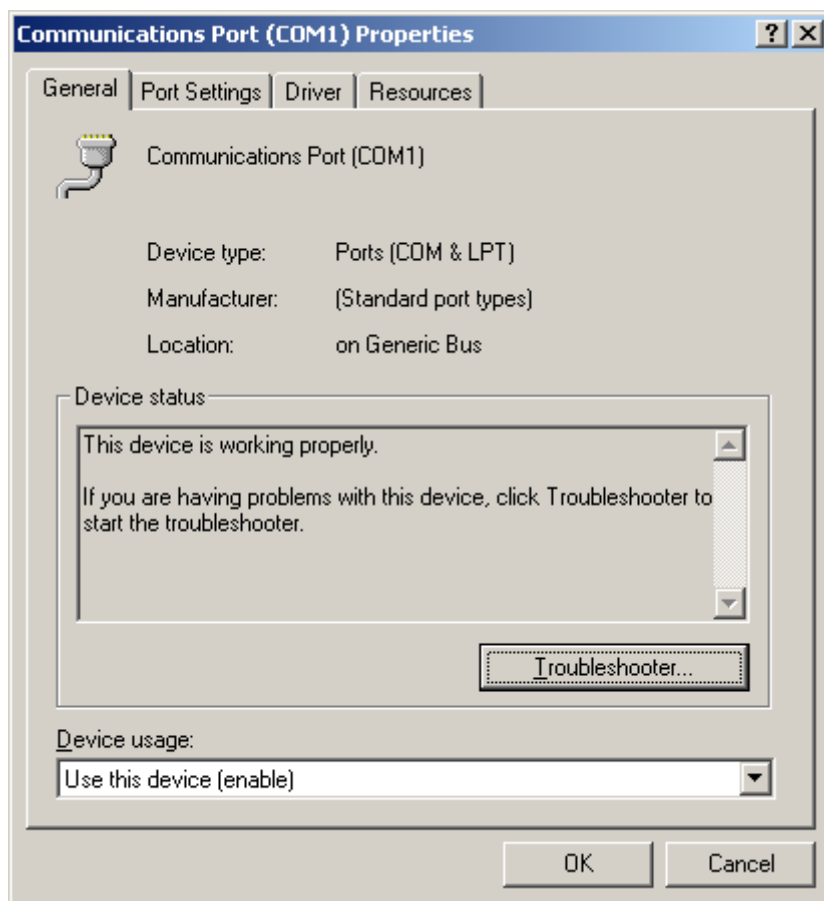
- 1 Select Start> Settings> Control Panel> System> Hardware> Device Manager:



2 Double click the symbol called "Ports (COM & LPT)".

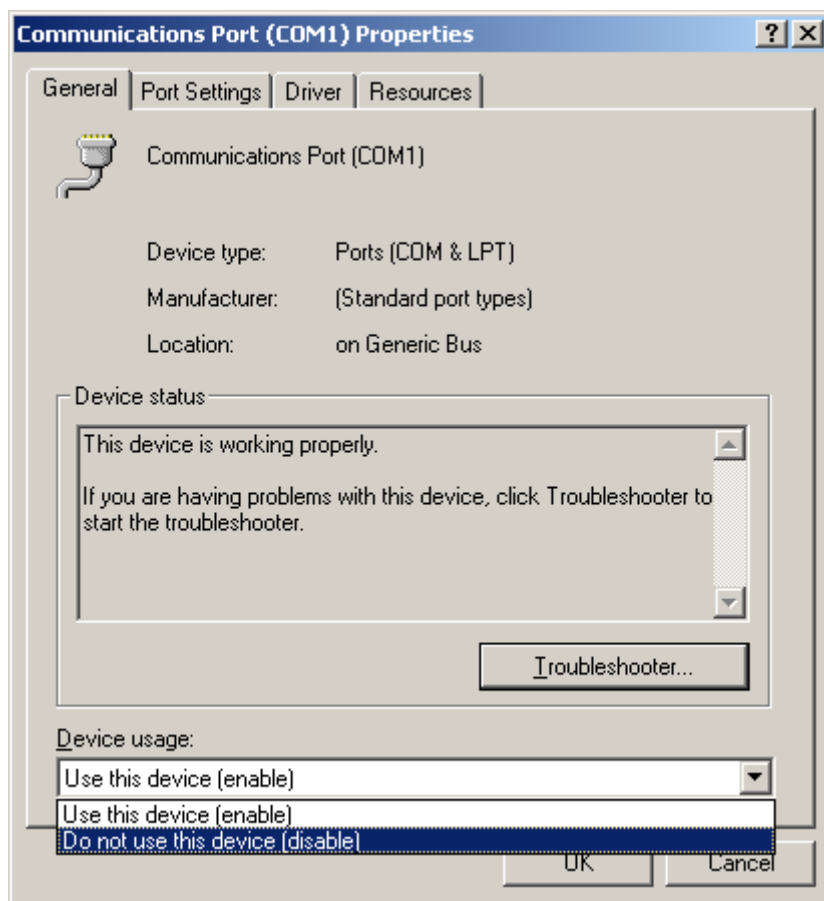


- 3 Double click the symbol called "Communication Port (COM1)" to open the Communication Port (COM1) Properties box:

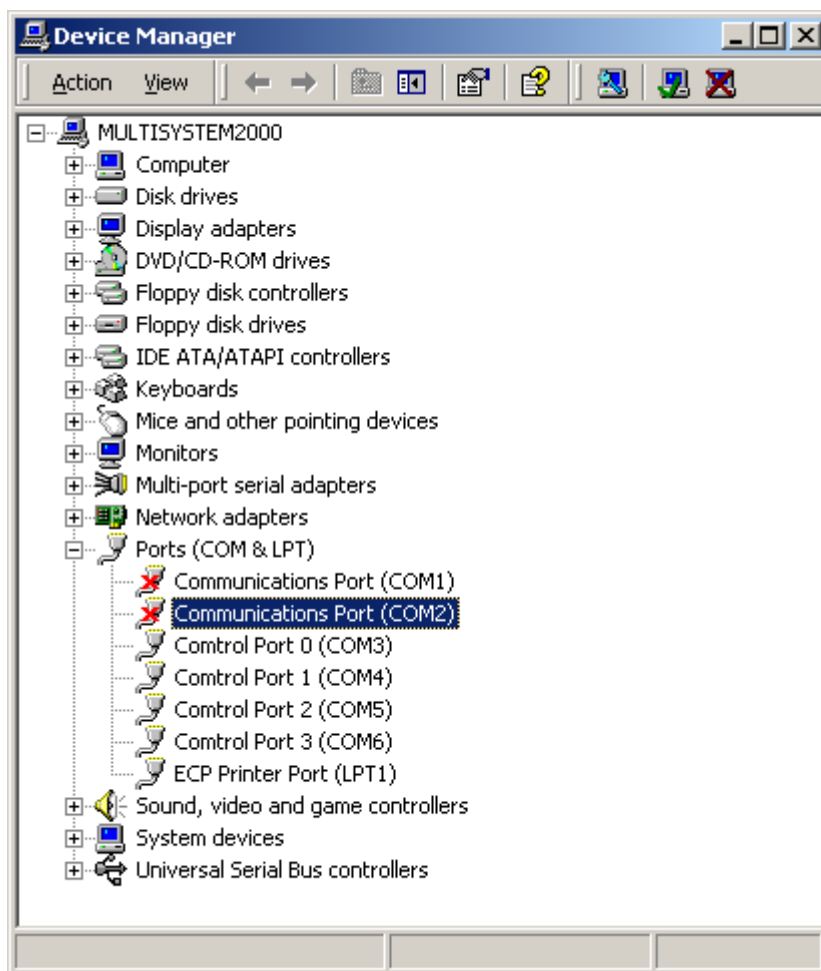




4 To disable the COM1 port select "Do not use this device (disable)" from the drop down menu:

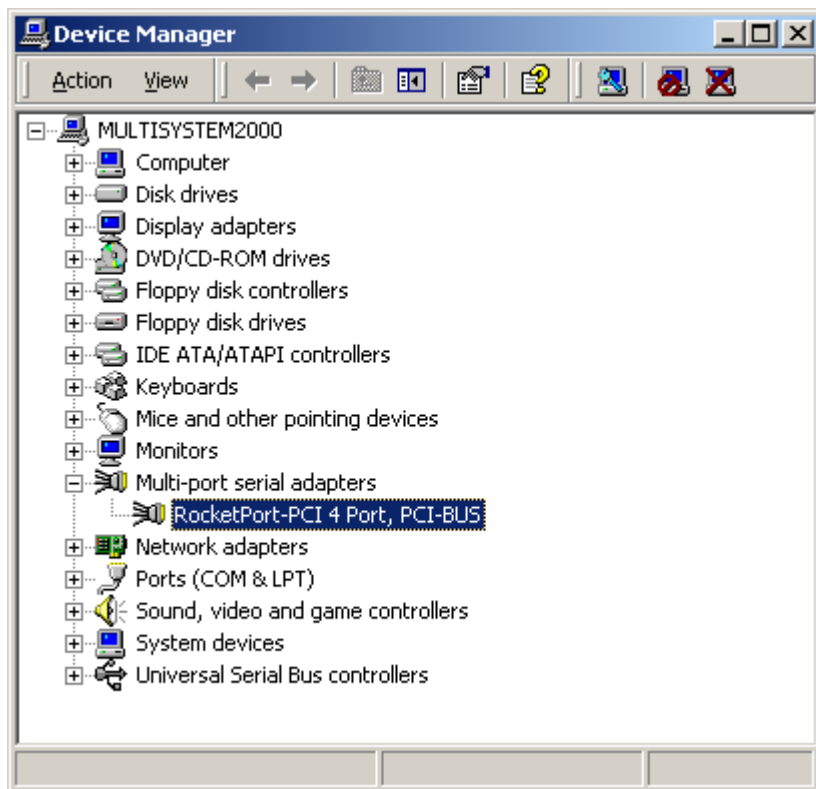


- 5 Repeat the previous steps to disable "Communication Port (COM2)". Return to the Device Manager:

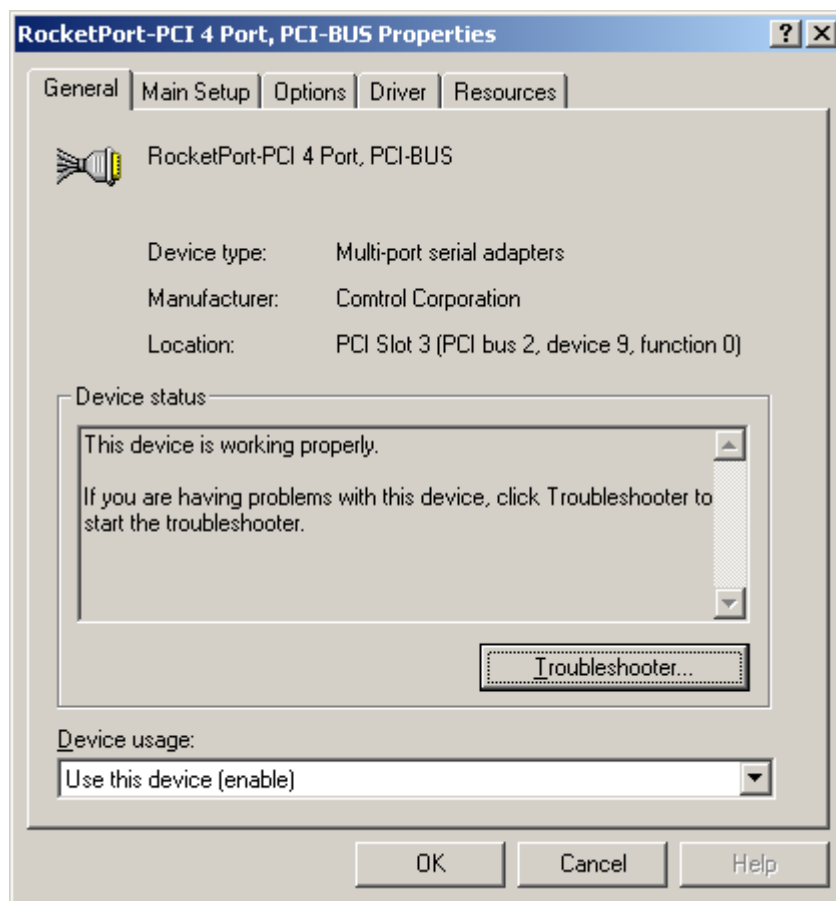


## Step2: Assigning COM1 to COM4 to the RocketPort Card

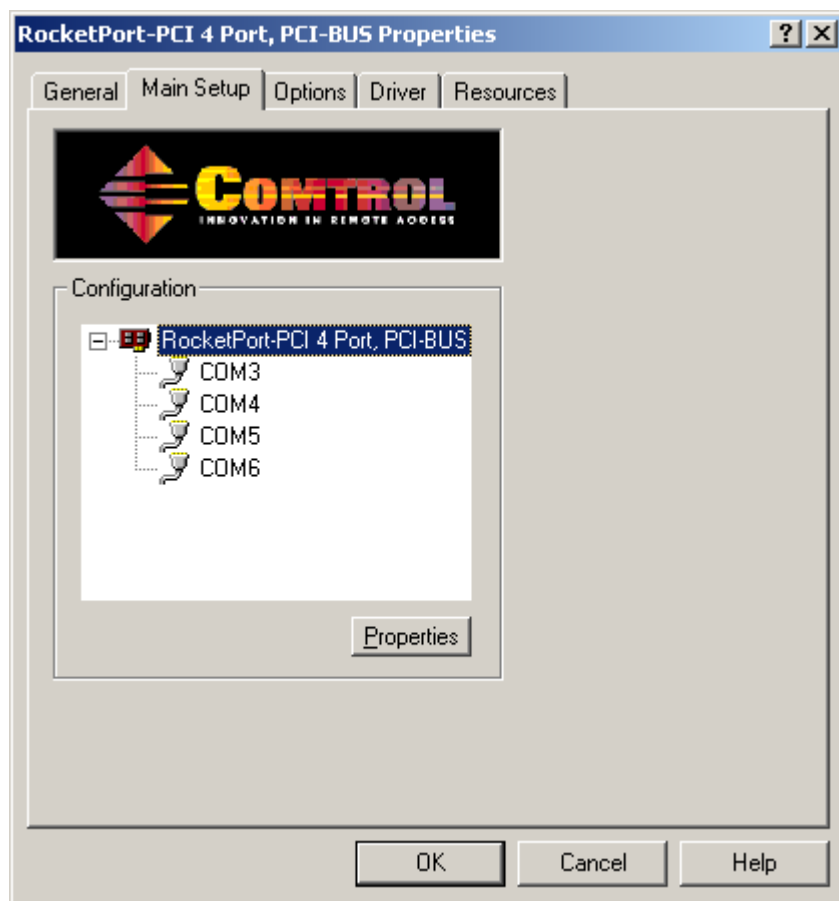
- 1 Select Start> Settings> Control Panel> System> Hardware> Device Manager. Double click the symbol called “Multi-port serial adapters”:



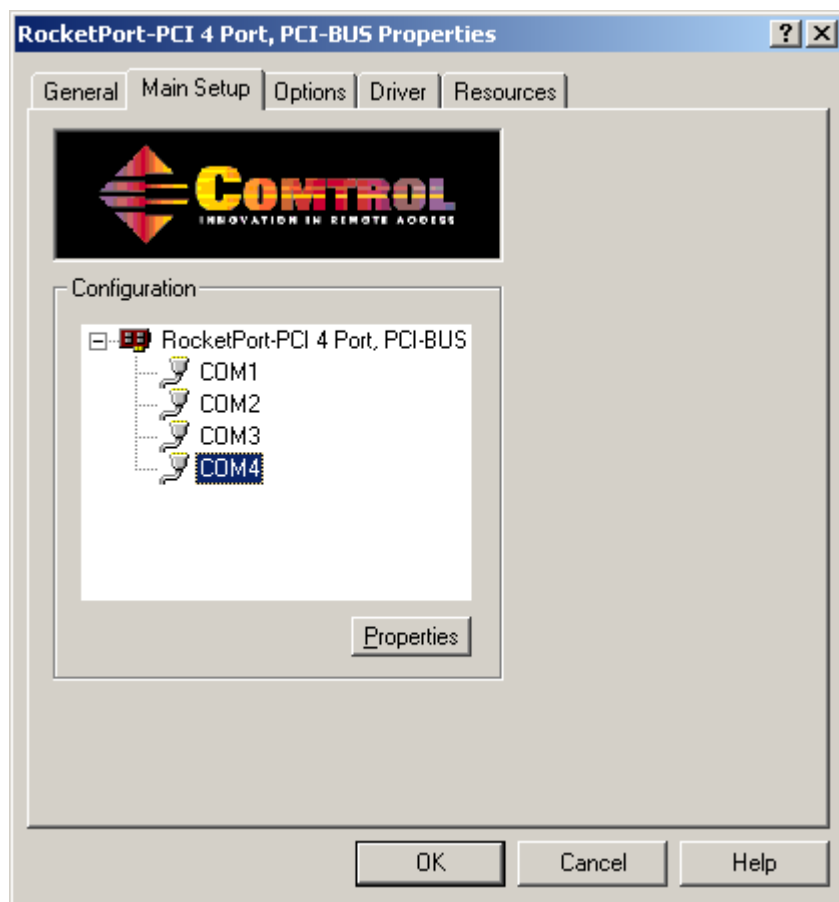
- 2 Double click the symbol called “RocketPort-PCI 4 Port, PCI-BUS” to open the RocketPort-PCI 4 Port, PCI-BUS Properties box:



3 Select "Main-Setup":



4 Alter the names from COMX to COM1....COM4:



5 Select "OK" to confirm the changes.

# MultiPort Card Setup Process (Windows NT)

The setup process of the MultiPort Card requires 2 steps:

Step 1: Disabling the standard serial ports

Step 2: Installing the RocketPort driver.

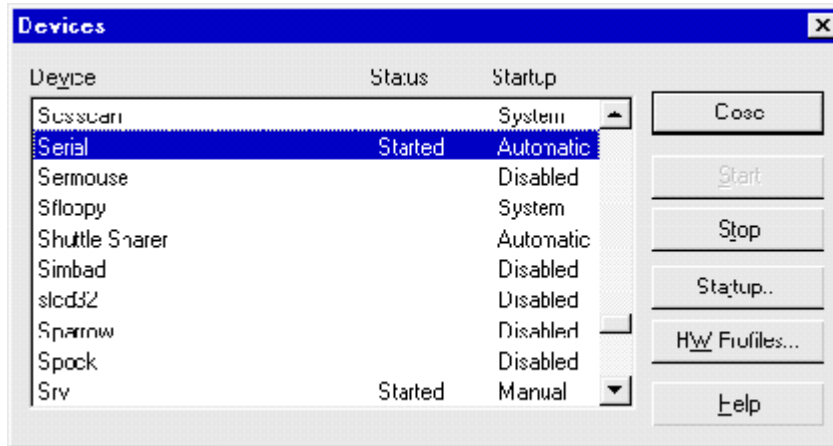
The setup process is described on the following pages. For support on configuring the RocketPort PCI/Quad DB9 Multi Port Serial card, please contact Agilent service personnel.

## Step 1: Disabling the Standard PC Serial Ports (COM1 and COM2)

The embedded serial ports of your PC must be disabled before you can use your RocketPort card. To do so,

- 1 open the *Control Panel* from the *Settings* menu.
- 2 Double click the symbol called "Devices" and look for the Device named "Serial". This is the standard device driver for the embedded serial ports.

- 3 To disable these serial ports press the Stop button to halt the active process (answer the following dialog with "Yes") and then change the startup mode from "Automatic" to "Disabled" (follow the instructions of the dialog presented after you have pressed the button "Startup...").
- 4 Close this dialog.



## Step 2: Installing the RocketPort PCI Driver

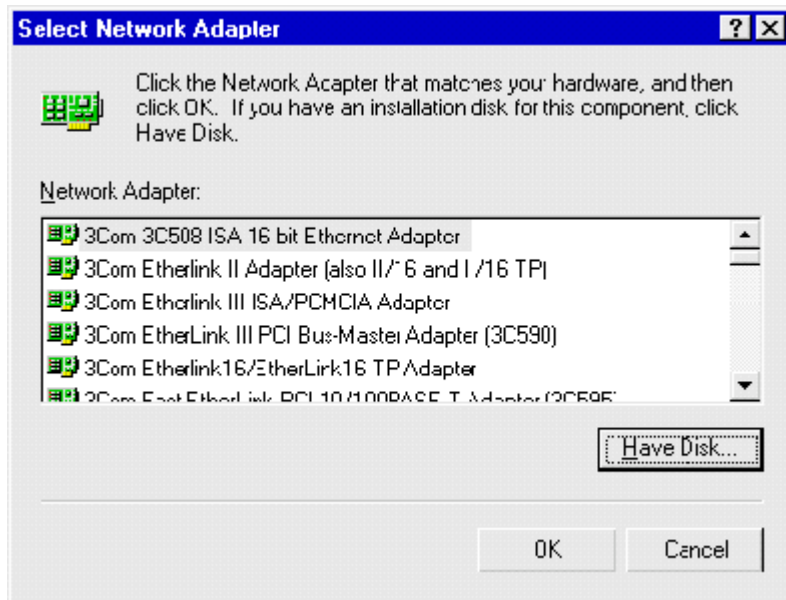
Installing the PCI Card as Network Adapter

The RocketPort card will be installed as an additional network adapter.

- 1 From the Control Panel open the "Network" dialog and click on the "Adapters" tab.
- 2 Press the "Add..." button to add a new Network Adapter.



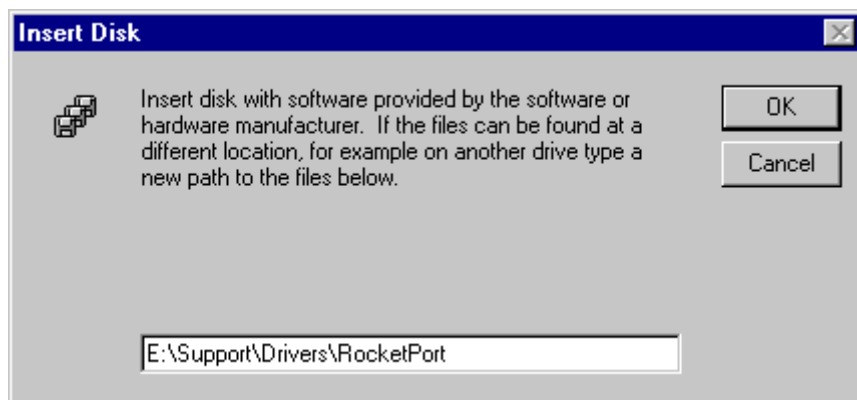
- 3 In the following dialog choose the "Have Disk..." - Button to load the RocketPort PCI/Quad driver.



- 4 Enter the path to the RocketPort driver.

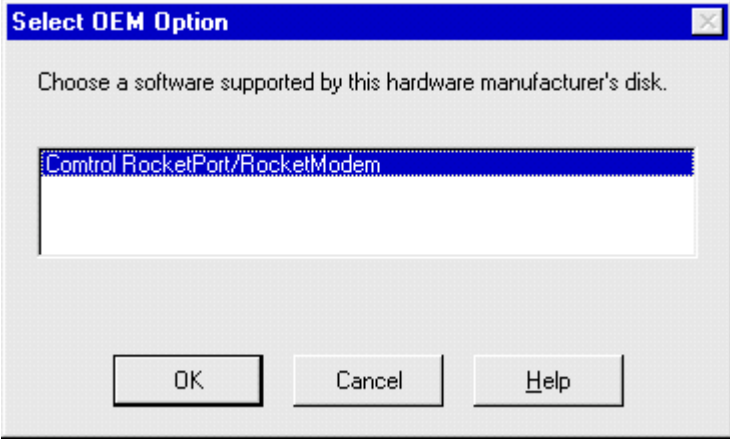
## NOTE

Use the RocketPort driver located on the bioanalyzer software CD. You can find the driver under: E:\Support\Drivers\RocketPort (where E is the letter of the CD-ROM drive).



In the next dialog you are able to choose the driver that should be installed (there should only be one choice, already pre-selected: *Control RocketPort/RocketModem*).

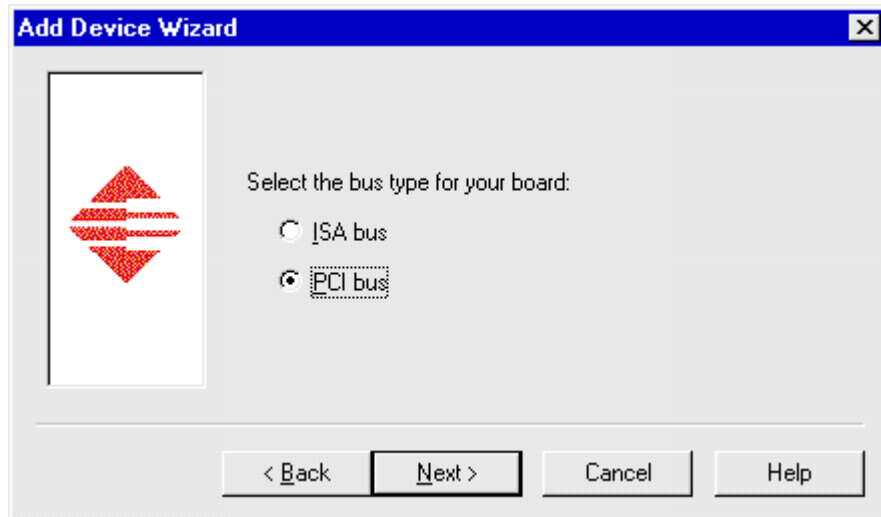
**5** Press *Ok* to start the installation.



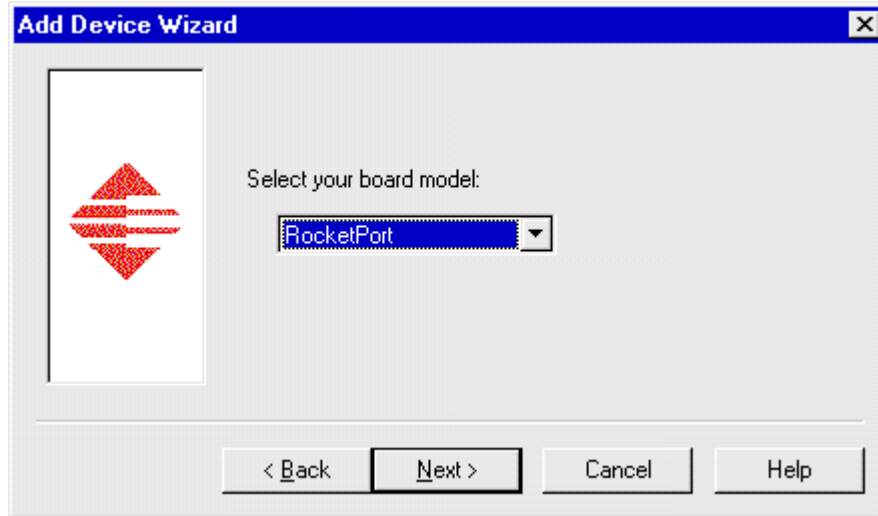
- 6** The device wizard will guide you through the rest of the setup. You can navigate between the different dialogs by clicking on the Back and Next buttons.



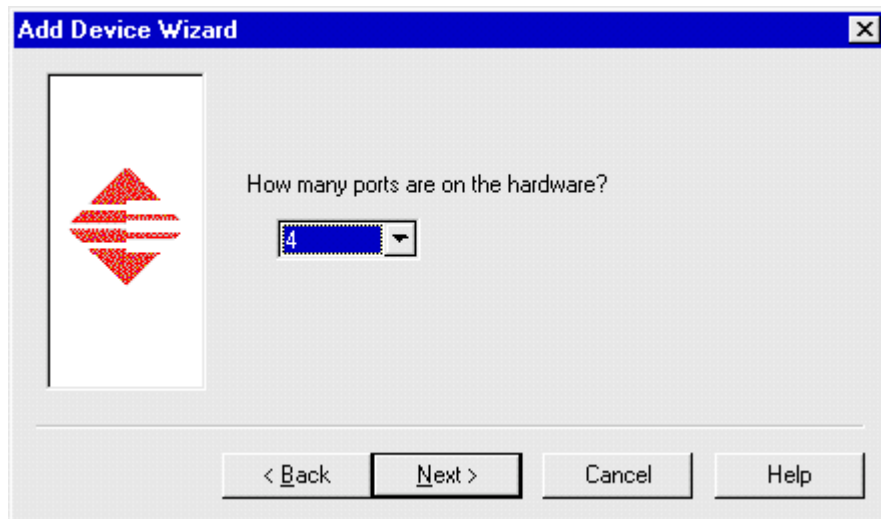
- 7** On the second window of the Add Device Wizard you must specify the bus type of the installed RocketPort card. Choose the option PCI bus.



- 8 Then you must specify the board model. You must keep the proposed setting: RocketPort.
- 9 Press Next to continue to the following dialogue.



- 10** The next step is to specify the number of ports supported by the installed card. Change the number of supported ports from 8 (default value) to 4 as this is the number of physical ports of the installed card.
- 11** Press Next to continue to the following dialogue.



**12** Click the "Finish" button to finish the installation.



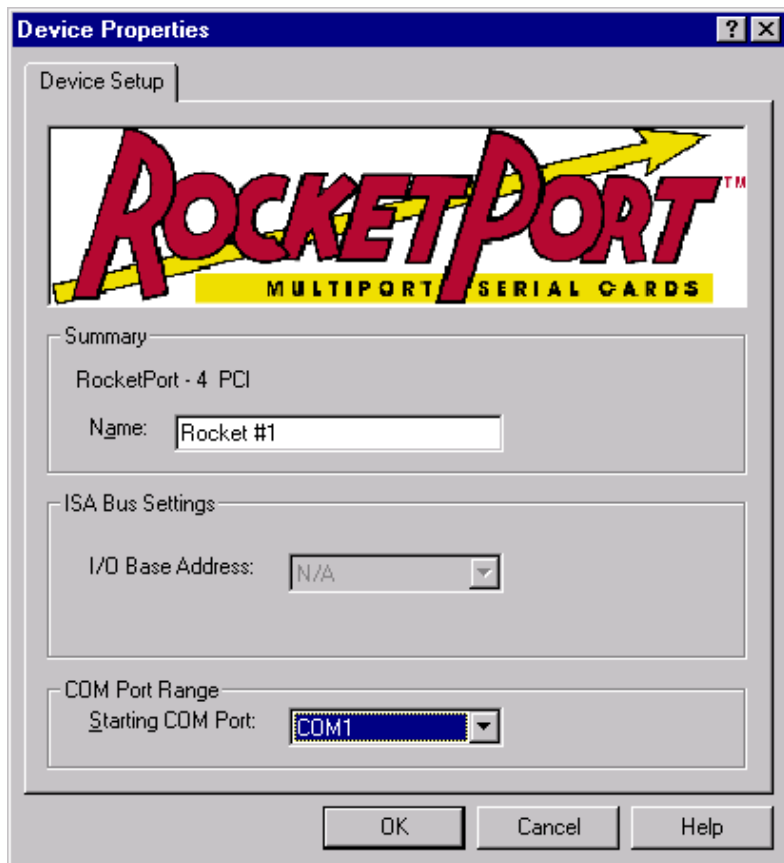
Now you have successfully installed the device driver for the RocketPort PCI/Quad DB9 card.



## Setup of the Device Properties

The installation process continues with the setup of the device properties.

- 1 You need to change the range of COM ports. Please change the *Starting COM Port* from *COM3* (default) to *COM1*.



- 2 Press *Ok* to save the settings. You can verify your specifications in the next dialog, which shows a summary of the RocketPort Setup.

## NOTE

Later on you can access this dialog by starting the Setup program of the RocketPort card from the **Start** menu.



3 Press OK to close the following window.



4 After closing the network setup window you will be requested to restart your computer.

5 Close any other open application and press Yes to reboot your computer.



After the reboot the RocketPort card is set up properly to work with the Agilent bioanalyzer 2100 Software. The instruments connected to the card can be referred as COM1 to COM4.

## NOTE

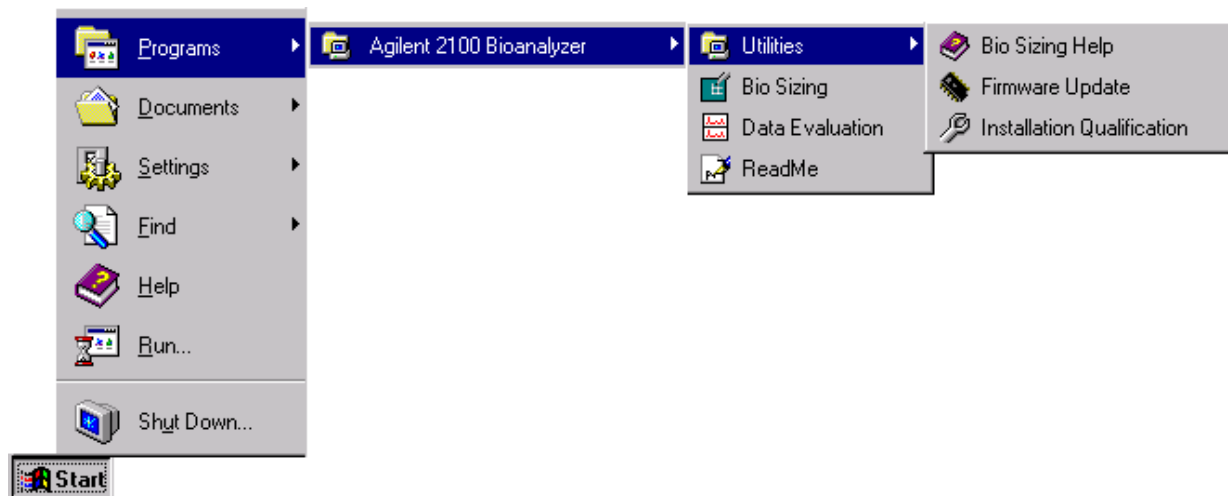
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The warning message "**Warning, PCI num-ports mismatch**" may appear in the event logbook of your PC. This is NOT an error and can be ignored. It happens only if you have no instrument connected to the RocketPort card. After connecting an instrument no event is logged after startup.

# Troubleshooting the Agilent 2100 Bioanalyzer Software

If your Agilent 2100 Bioanalyzer software is no longer working properly, you can check for corrupted or missing files.

- 1 If the Bio sizing is running, close it.
- 2 Start the software test tool by clicking Installation Qualification in the Agilent 2100 Bioanalyzer program group.



- 3** The Installation Qualification interface appears. Click Start Validation to start the software test tool. The result of the installation qualification test depends on whether the software installation is complete and no files are corrupted.



- 4** If the test passes and the Agilent 2100 Bioanalyzer system still does not function correctly, see **Communication—14** and **Hardware Diagnostics—48** for further troubleshooting procedures. Finally, check your application, see **Troubleshooting the DNA Application—52**, **Troubleshooting the RNA Application—123** or **Troubleshooting the Protein Application—183**.
- 5** If the test fails, reinstall the Agilent 2100 Bioanalyzer software using the Agilent 2100 Bioanalyzer software CD-ROM that is supplied with the system.
- Insert the Agilent 2100 Bioanalyzer software CD-ROM in the CD-ROM drive of your PC.
  - Start Windows NT Explorer and go to the CD-ROM drive.

- Double-click on the SETUP.EXE file and follow the instructions on the screen.
  - Repeat steps 1 through 4 to verify proper installation.
- 6** If the test continues to fail, save the result of the test by choosing File > Save log file as... in the Installation Qualification interface and call Agilent Technologies.

# Hardware Diagnostics

## Built in Tests—Firmware Error Messages

Whenever you start a run, the firmware of the Agilent 2100 Bioanalyzer checks for errors such as, for example, defective high voltage supplies, or missing conductivity between wells. If an error is detected, the firmware pops up a message box and aborts the run. Further, the message box contains hints on how to resolve the problem, or tells you to call Agilent Technologies.

## Manual Tests

To successfully perform the complete set of hardware diagnostic tests, you need a new chip. With the chip and a diagnosis software interface, you can run system component tests and check all hardware components of the Agilent 2100 Bioanalyzer. Here is a complete list of hardware diagnostic tests.

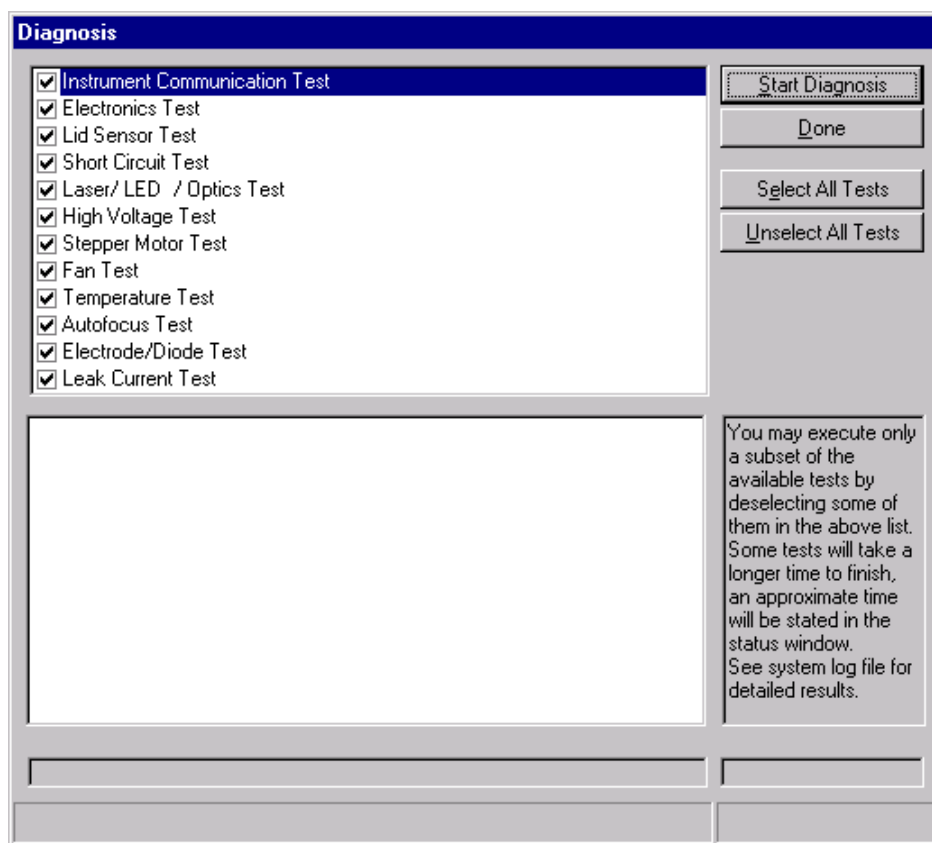
Test	Description
Instrument communication test	Checks for proper communication between PC and Agilent 2100 Bioanalyzer.
Electronic test	Verifies proper functioning of all electronic boards in the Agilent 2100 Bioanalyzer.
Lid sensor test	Checks for the devices sensing for open or closed lid, and for laser and LED off when lid is being closed.
Short circuit test	Checks for instrument leak currents.



Test	Description
Laser / LED / Optics.	Checks for proper alignment of internal optics and proper function of the laser and LED
High voltage test	Checks the calibration of all 16 high voltage power supplies in the Agilent 2100 Bioanalyzer.
Stepper motor test	Checks for proper movement of stepper motor.
Fan test	Checks that the fan is running at the appropriate speed.
Temperature test	Checks that the temperature ramp up speed of the heater plate is within specifications.
Autofocus test	Checks focusing capability of optical system.
Electrode/diode test	Checks photodiode and current versus voltage performance of Agilent 2100 Bioanalyzer system.
Leak current test	Measures electrode cartridge leak current(s) between pins.

## Test Procedure:

- 1 Access the hardware diagnostic tests by selecting Tools and then Diagnose Instrument in the Agilent 2100 Bioanalyzer software.
- 2 Select any of the hardware tests you want to apply from the list given in the interface. The recommendation is to apply all tests.



- 3 Click the Start diagnosis button to execute the tests.

## NOTE

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If there is no communication between the Agilent 2100 Bioanalyzer and the PC, the software will prompt you. See Troubleshooting the Instrument Communication—14 for troubleshooting hints.

- 4 Follow the instructions as given by the Agilent 2100 Bioanalyzer software.
- 5 At the end of the procedure all tests must be passed.
- 6 If the tests are not passed, redo the tests.
- 7 If failures still persist, call Agilent Technologies.


# Troubleshooting the DNA Application

## Essential Measurement Practices

For hints on how to handle chips and chemicals, see **Essential Measurement Practices—7**.

## Troubleshooting the Application

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Click the  or  button next to the error message to view a help screen that is specific for that error.

Additional information regarding the nature of a problem can often be found in the Run Log for the data file. Choose Tools > View Log File > Run Log. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by your Agilent 2100 Bioanalyzer might not be what you expected. To help you find the reason for the discrepancy, see **Symptoms—54**.

For most observations you will find at least one corresponding example, depicting a typical electropherogram or result table. Once you have identified the observation that resembles the outcome of your experiment, you will get a set of assigned causes listed by priority.

The causes are grouped into three levels:

- **most probable cause**
- **probable cause**
- **least probable cause**

A list of solutions that help you to fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by priority.

If you are not able to assign a symptom to your problem, compare your electropherogram with the **List of DNA Electropherograms—106**.

# Symptoms

Click the icon to see an example, or go straight to the troubleshooting hints.

Clogged Spin Filters—56

Too High Quantification Results—57

Too Low Quantification Results—58

Wrong Sizing Result—59

Poor Chip Performance—61

Chip Not Detected—62



Additional Sample or Ladder Peaks—63



Spikes/Glitches—66



Poor Sensitivity—69



Noisy Electropherogram—72



Broad Peaks—74



Missing Peaks or Marker Peaks—77



Poor Baseline: Dips—80



Poor Baseline: Drift—82



Poor Baseline: Noise—85



Poor Baseline: Jumps—88



Poor Baseline: Bend—90



No Peaks and High Background—92



No Peaks and Low Background—95



Cross Contamination—98



Peaks Migrating Late—100



Late Migration and Peak Broadening—102



Bend Ladder Baseline—104

List of DNA Electropherograms—106

# Clogged Spin Filters

Most Probable Causes	Solution
Gel-dye mix was centrifuged at too low g-value.	Refer to the DNA Reagent Kit Guide for proper centrifuge settings.
Cooled centrifuge was used for preparation of gel-dye mix.	Repeat centrifugation step without cooling.
Probable Causes	Solution
Particles in the gel-dye mix.	Repeat the preparation of the gel-dye mix. Wear powder-free gloves only.

Back to **Symptoms**



# Too High Quantification Results

Most Probable Causes	Solution
Pipetting error during preparation of mixtures.	Check dilution procedure.  Check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid.  Use appropriate pipette and tips.
Probable Causes	Solution
Dye concentration too low (marker disappears).	Use dye concentration according to the DNA Reagent Kit Guide. Let the dye warm up to room temperature before preparing the gel-dye mix.
Least Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.

Back to **Symptoms**

# Too Low Quantification Results

Most Probable Causes	Solution
Pipetting error during preparation of mixtures.	Check dilution procedure.  Check calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid.  Use appropriate pipette and tips.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use the IKA vortexer. Adjust the speed to the set-point.
Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Sample concentration too high.	Use sample concentration according to the DNA Reagent Kit Guide.
Least Probable Causes	Solution
Dye concentration too high.	Use dye concentration according to the DNA Reagent Kit Guide. Let the dye warm up to room temperature before preparing the gel-dye mix.

Back to **Symptoms**

# Wrong Sizing Result

Most Probable Causes	Solution
DNA ladder degraded.	Check expiration date of chemicals.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ). Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
No ladder in ladder well.	Use a new chip.

Least Probable Causes	Solution
Vibration of Agilent 2100 Bioanalyzer.	<p>Don't touch Agilent 2100 Bioanalyzer during a run.</p> <p>Remove vibration devices, such as vortexers and vacuum pumps, from bench.</p>
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 Bioanalyzer in thermally stable environment.
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> . If the power supply is defective, call Agilent Technologies.

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# Poor Chip Performance

Most Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Probable Causes	Solution
Chip preparation was done with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature before use.
Chips were stored in the fridge/freezer.	Prepare a new chip. Store chips at room temperature.
Amount of liquid pipetted is too low or chip is empty.	Check assay procedure on amount of liquid to be pipetted.
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> . If the power supply is defective, call Agilent Technologies.

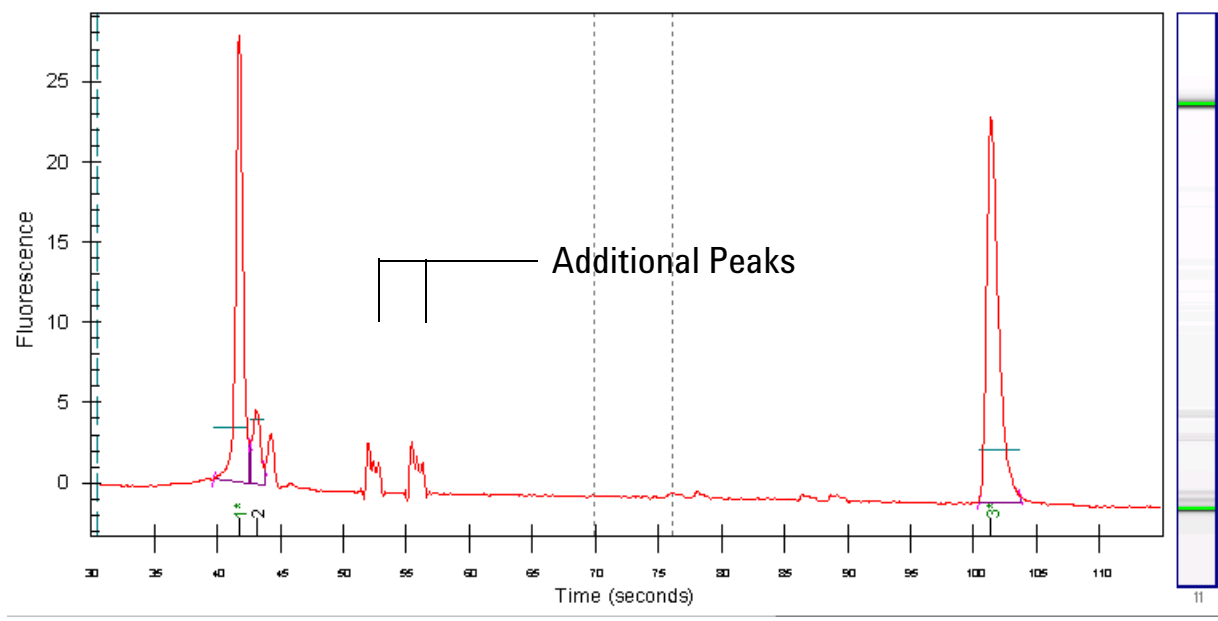
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# Chip Not Detected

Most Probable Causes	Solution
Amount of liquid pipetted is too low or chip is empty.	Check assay procedure on amount of liquid to be pipetted. Pipette sample or buffer in all wells.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Probable Causes	Solution
No communication between Agilent 2100 Bioanalyzer and PC.	Check instrument communication like described under <b>Troubleshooting the Instrument Communication—14</b> .
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> . If the power supply is defective, call Agilent Technologies.

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# Additional Sample or Ladder Peaks



Show me how to solve Additional Sample or Ladder Peaks

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# Additional Sample or Ladder Peaks

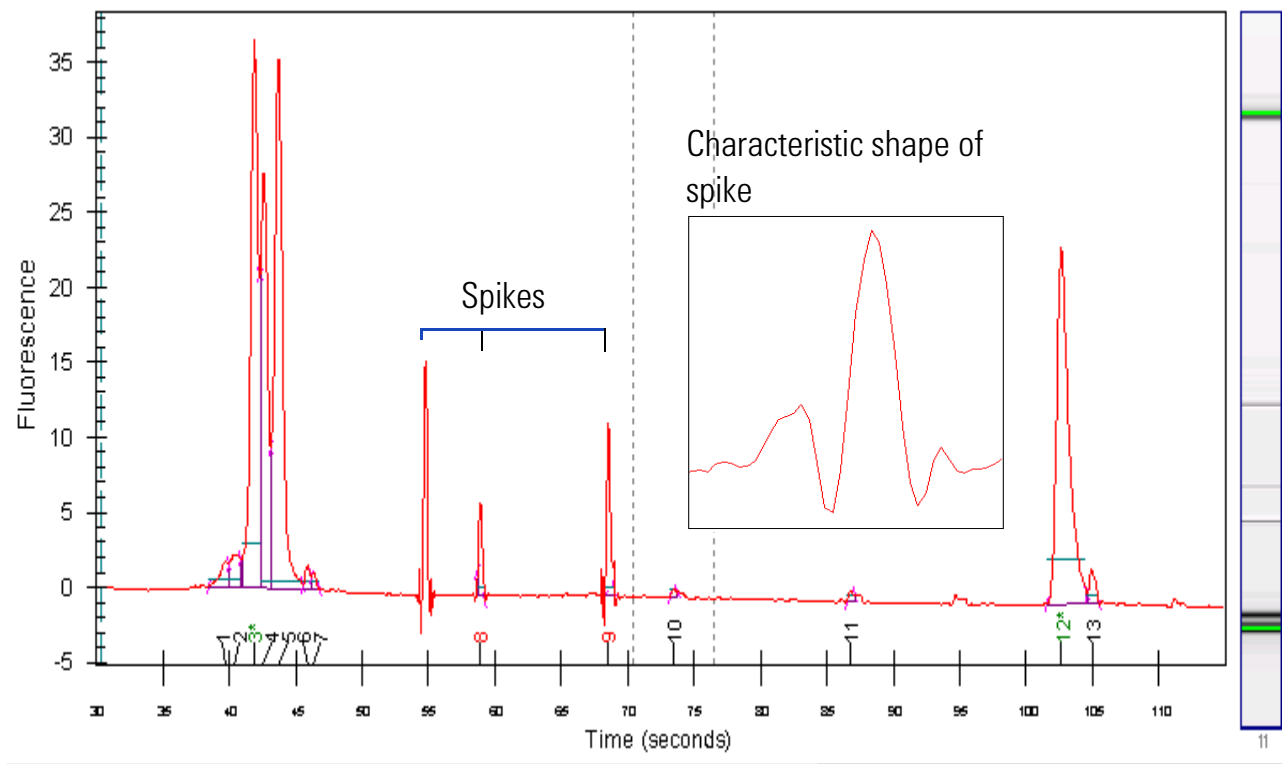
Most Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only.
Dust particles in separation channels.	Don't touch the wells of the chip.  Clean the electrodes.  Load the chip immediately after taking it out of its sealed bag.
Particles of protective foam pad on electrode cartridge.	Make sure to remove foam particles of the electrode cartridge before use.
Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run.  Remove vibration devices, such as vortexers and vacuum pumps, from bench.



Least Probable Causes	Solution
DNA ladder degraded.	Check expiration date of chemicals.

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# Spikes/Glitches



Show me how to solve **Spikes/Glitches**

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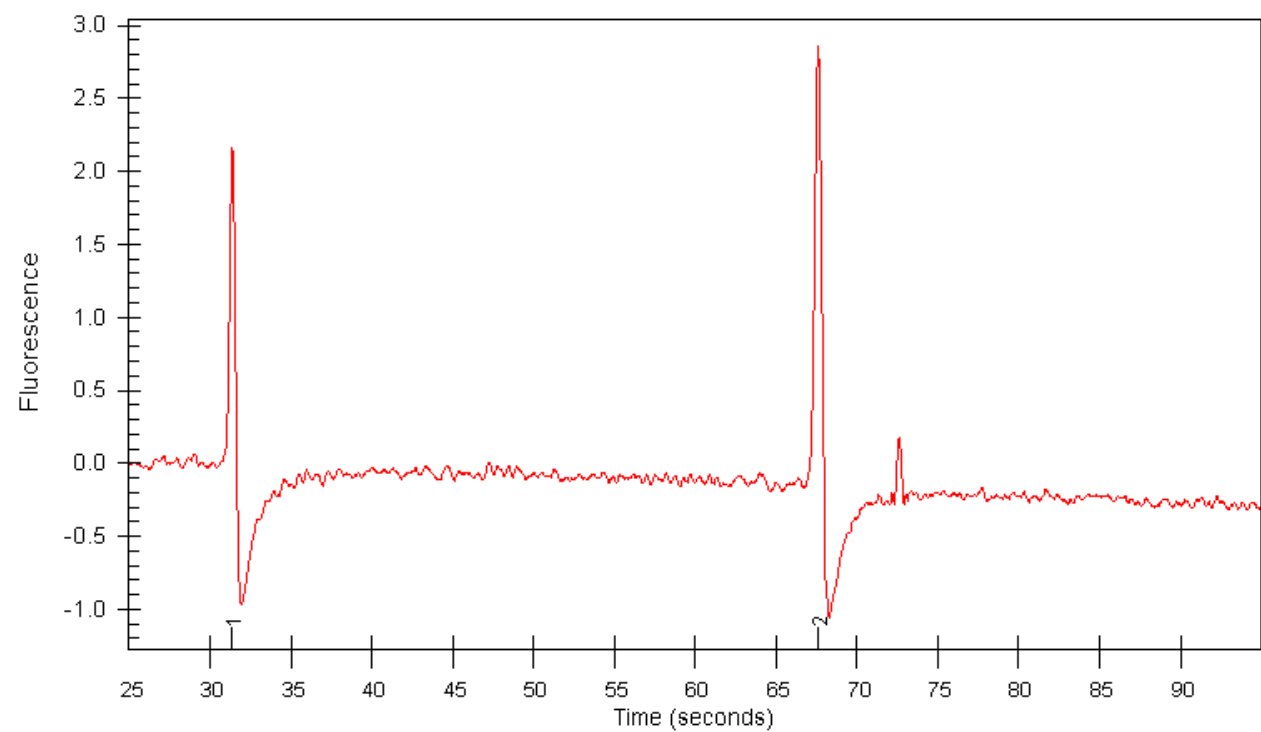
# Spikes/Glitches

Most Probable Causes	Solution
Chip/gel-dye mix contaminated.	Prepare new chip with new gel-dye mix:  Wear powder-free gloves only.  Don't touch the underside of the chip.  Don't touch the wells of the chip.  Clean the electrodes.  Load the chip immediately after taking it out of its sealed bag.
Gel-dye mix not properly prepared.	Refer to the Reagent Kit Guide for proper preparation of the gel-dye mix. Let the dye warm up to room temperature before preparing the gel-dye mix.
Chip not properly prepared.	Prepare a new chip. Allow all reagents and samples to warm up to room temperature before use.

Probable Causes	Solution
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Least Probable Causes	Solution
Power outlett	Install power filter.

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# Poor Sensitivity



Show me how to solve **Poor Sensitivity**

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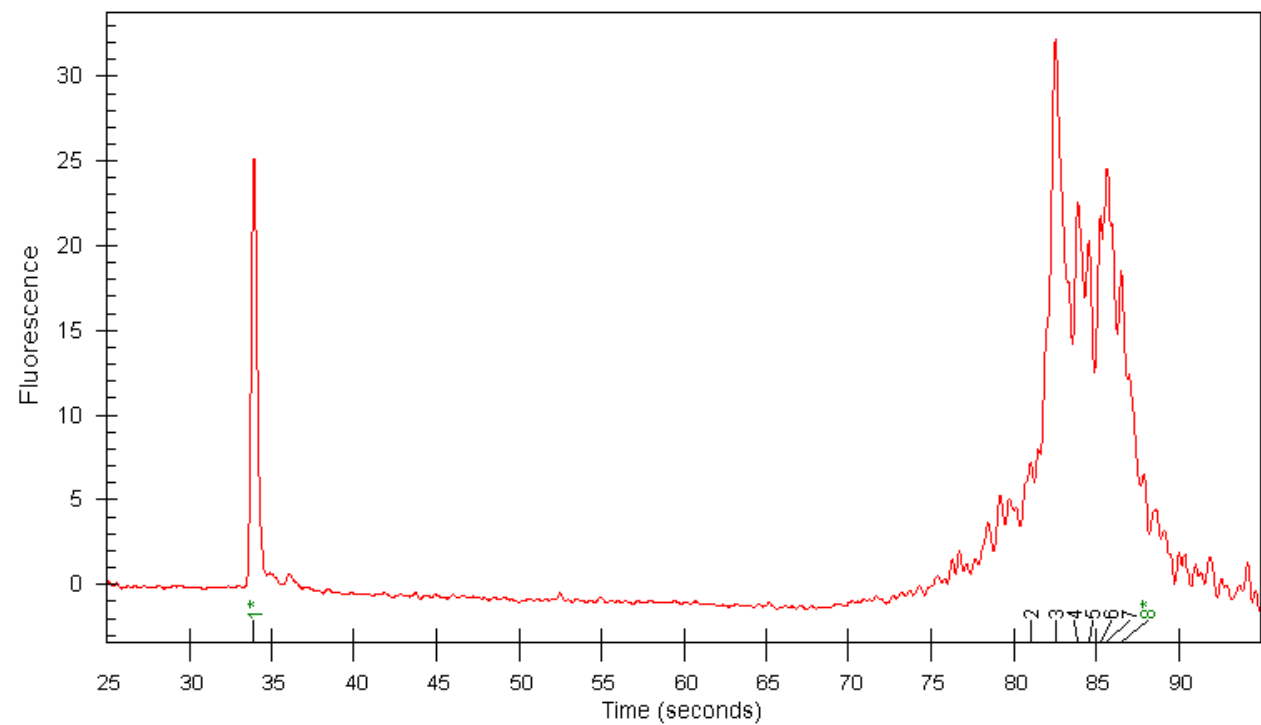
# Poor Sensitivity

Most Probable Causes	Solution
Dye concentration too low (marker disappears).	Use dye concentration according to the Reagent Kit Guide.  Let the dye warm up to room temperature before preparing the gel-dye mix.
Pipetting error during preparation of mixtures.	Check dilution procedure.  Check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Holding the pipette at a slight angle will ensure proper dispensing of the liquid.  Use appropriate pipette and tips.
Probable Causes	Solution
Fingerprint on focusing lens.	Clean lens like decribed in <b>Lens Maintenance—270</b> .
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use IKA shaker for chip vortexing. Adjust speed to set-point.

Least Probable Causes	Solution
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
Vibration of Agilent 2100 Bioanalyzer.	<p>Don't touch Agilent 2100 Bioanalyzer during a run.</p> <p>Remove vibration devices, such as vortexers and vacuum pumps, from bench.</p>
Autofocus failure.	<p>Check autofocus using the <b>Hardware Diagnostics—48</b>. If autofocus fails, call Agilent Technologies.</p>
Laser defective.	<p>Check laser using the <b>Hardware Diagnostics—48</b>.</p> <p>If the laser is defective, call Agilent Technologies.</p>

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# Noisy Electropherogram



Show me how to solve Noisy Electropherogram

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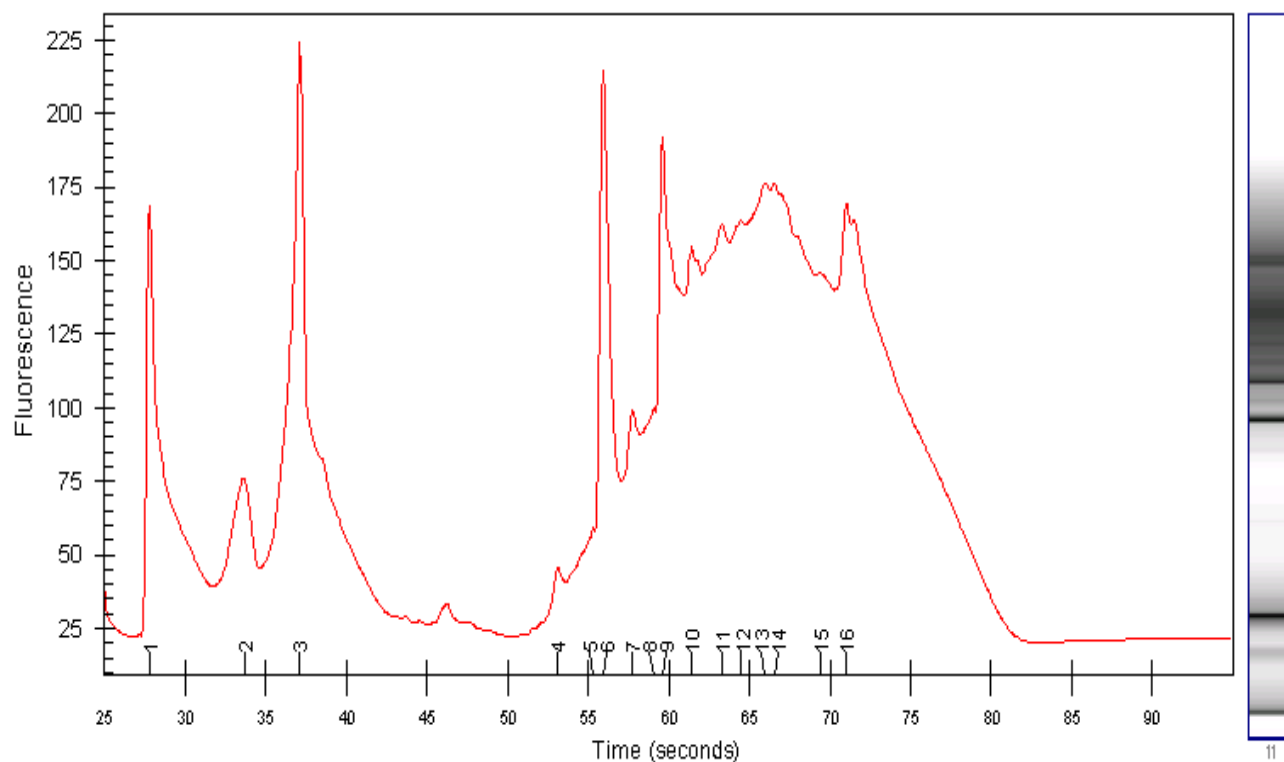


# Noisy Electropherogram

Most Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	<p>Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b>).</p> <p>Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).</p>
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
Vibration of Agilent 2100 Bioanalyzer.	<p>Don't touch Agilent 2100 Bioanalyzer during a run.</p> <p>Remove vibration devices, such as vortexers and vacuum pumps, from bench.</p>

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## Broad Peaks



Show me how to solve **Broad Peaks**,  
see also **Late Migration and Peak Broadening**

## Back to Symptoms

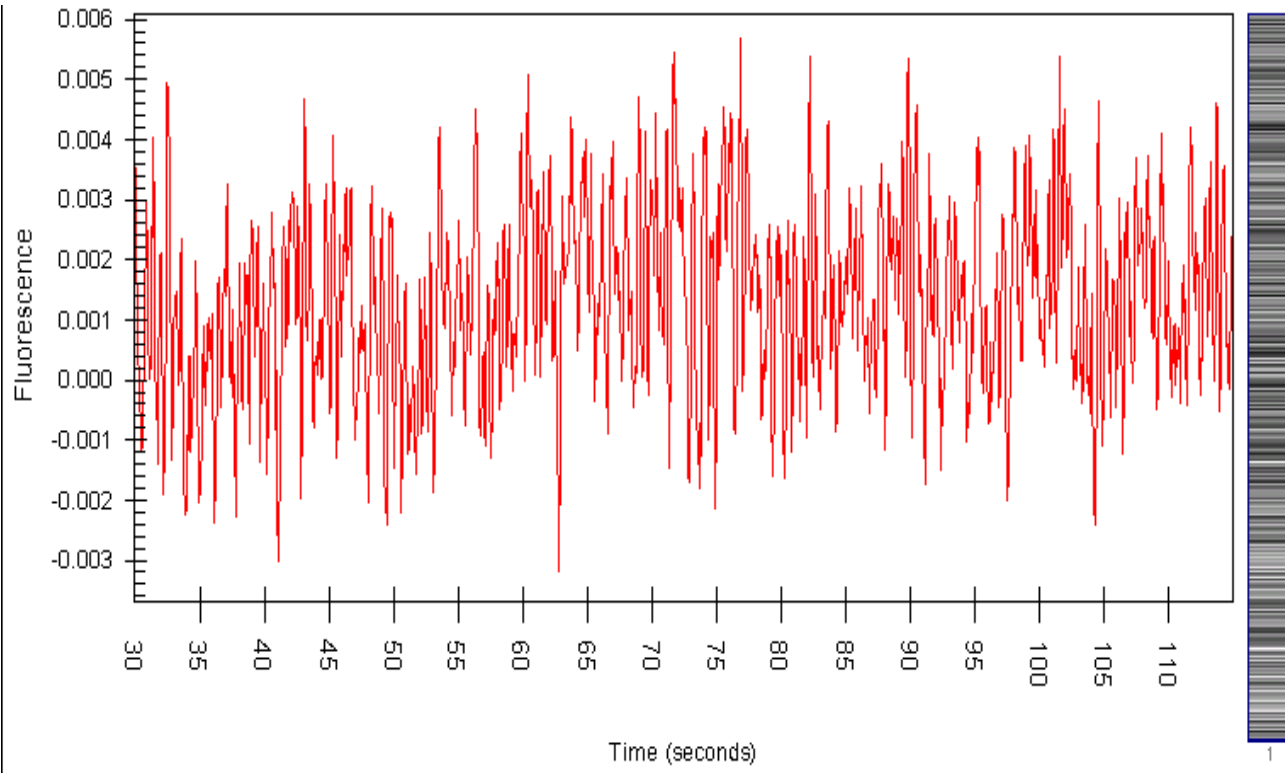
# Broad Peaks

Most Probable Causes	Solution
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b>  Replace electrode cartridge.
Clogged gasket and plastic adapter of priming station	Perform seal test like described in <b>Maintaining the Chip Priming Station—271</b> . Clean/replace gasket and plastic adapter if necessary.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Dye concentration too high.	Use dye concentration according to the Reagent Kit Guide.  Let the dye warm up to room temperature before preparing the gel-dye mix.
Particles of protective foam pad on electrode cartridge.	Make sure to remove foam particles of the electrode cartridge before use.

Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> . If the power supply is defective, call Agilent Technologies.

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# Missing Peaks or Marker Peaks



Show me how to solve **Missing Peaks or Marker Peaks**

Back to **Symptoms**

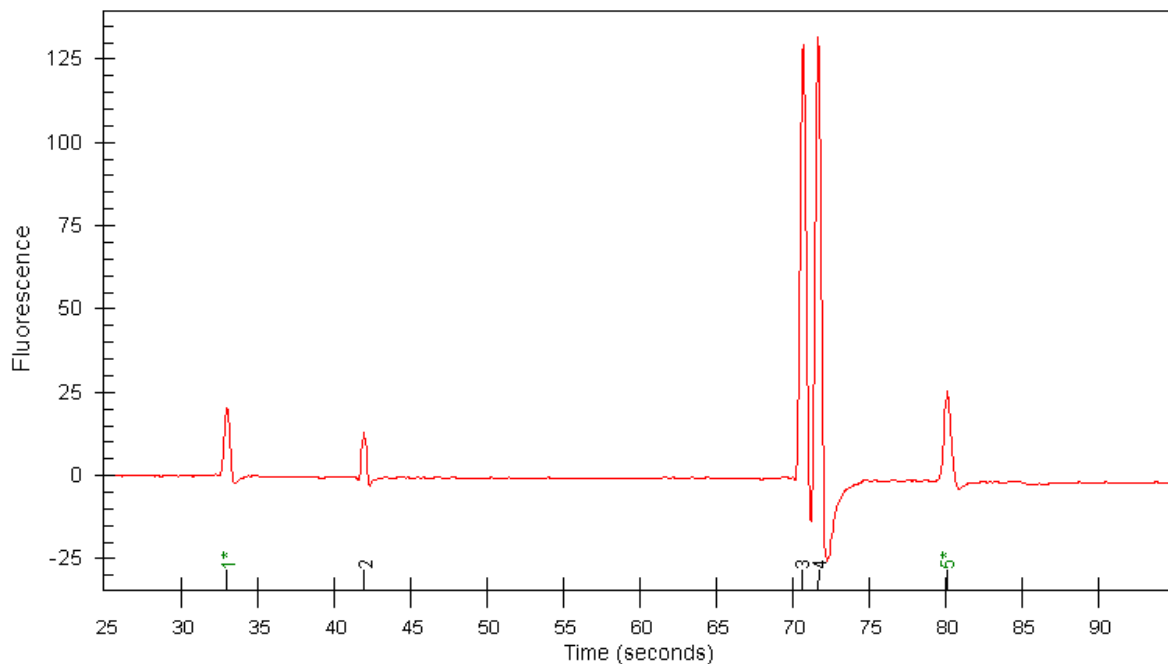
# Missing Peaks or Marker Peaks

Most Probable Causes	Solution
Laser broken.	Perform Laser/LED/Optics test and Autofocus test like described in <b>Hardware Diagnostics—48</b> . If tests fail call Agilent Technologies.
No ladder/samples in wells.	Use a new chip. Pipette ladder/sample in all wells
Clogged priming station.	Check the performance of the chip priming station by applying the seal test. For details see <b>Maintaining the Chip Priming Station—271</b> .
Wrong settings of chip priming station.	For the correct position of the syringe clip and base plate please refer to the Reagent Kit Guide.
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b>  Replace electrode cartridge.
Probable Causes	Solution
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Holding the pipette at a slight angle will ensure proper dispensing of the liquid.  Use appropriate pipette and tips.

Least Probable Causes	Solution
Autofocus failure.	Check autofocus by means of the <b>Hardware Diagnostics—48</b> . If autofocus fails, call Agilent Technologies.
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> .  If the power supply is defective, call Agilent Technologies.

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## Poor Baseline: Dips



Show me how to solve **Poor Baseline: Dips**

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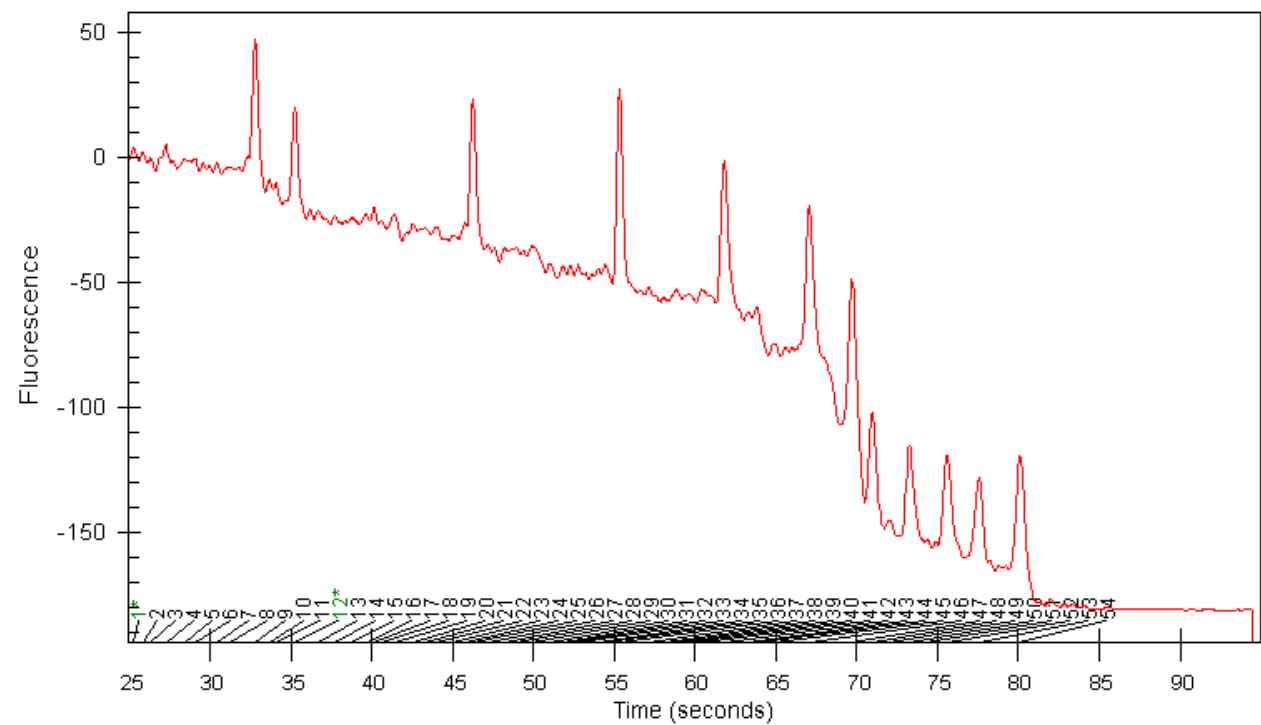


# Poor Baseline: Dips

Probable Causes	Solution
Too high sample concentration.	Use sample concentration according to the DNA Reagent Kit Guide.
Sample impurities: e.g. genomic DNA, ss DNA, etc.	Check DNA-isolation protocol. If possible, clean up samples.
Least Probable Causes	Solution
Autofocus failure.	Check autofocus by means of the <b>Hardware Diagnostics—48</b> . If autofocus fails, call Agilent Technologies.

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# Poor Baseline: Drift



Show me how to solve **Poor Baseline: Drift**

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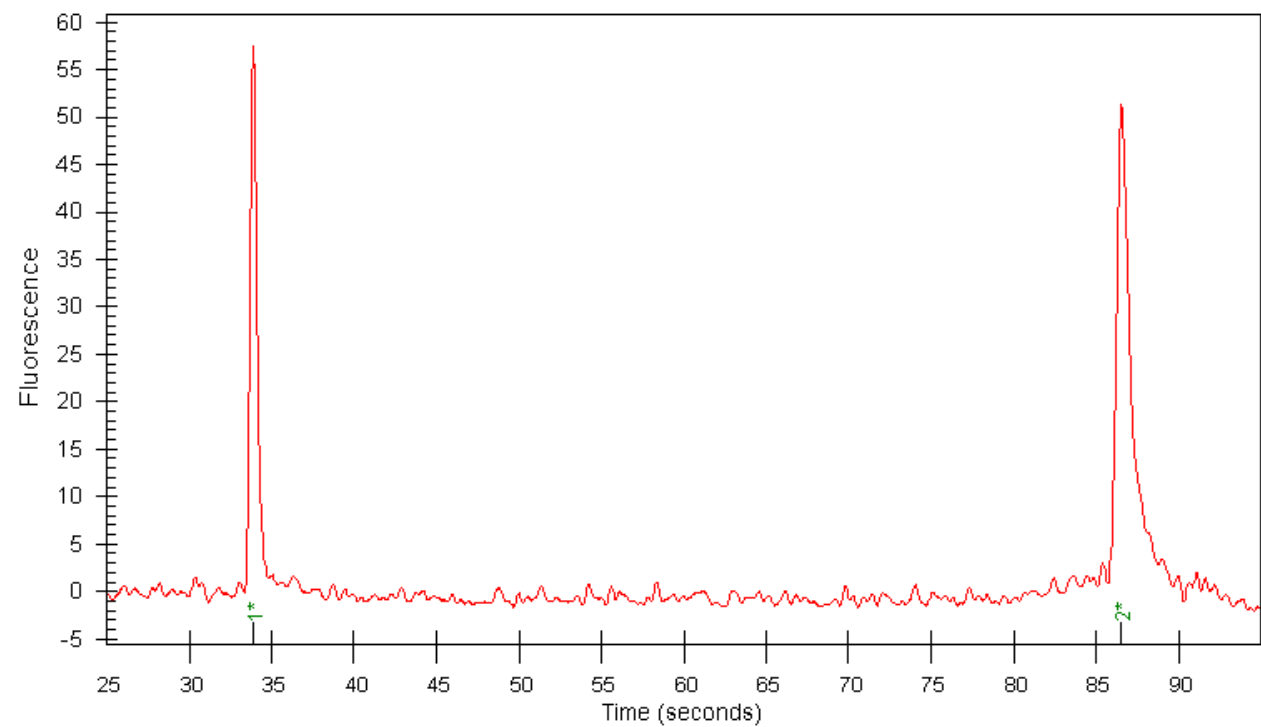
# Poor Baseline: Drift

Most Probable Causes	Solution
Leak current due to dirty electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b> .  Replace electrode cartridge.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Dye concentration too low.	Use dye concentration according to the Reagent Kit Guide.  Let the dye warm up to room temperature before preparing the gel-dye mix.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Clogged gasket and plastic adapter of priming station	Perform seal test like described in <b>Maintaining the Chip Priming Station—271</b> . Clean/replace gasket and plastic adapter if necessary.

Probable Causes	Solution
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
Least Probable Causes	Solution
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 Bioanalyzer in thermally stable environment.
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> . If the power supply is defective, call Agilent Technologies.

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# Poor Baseline: Noise



Show me how to solve **Poor Baseline: Noise**

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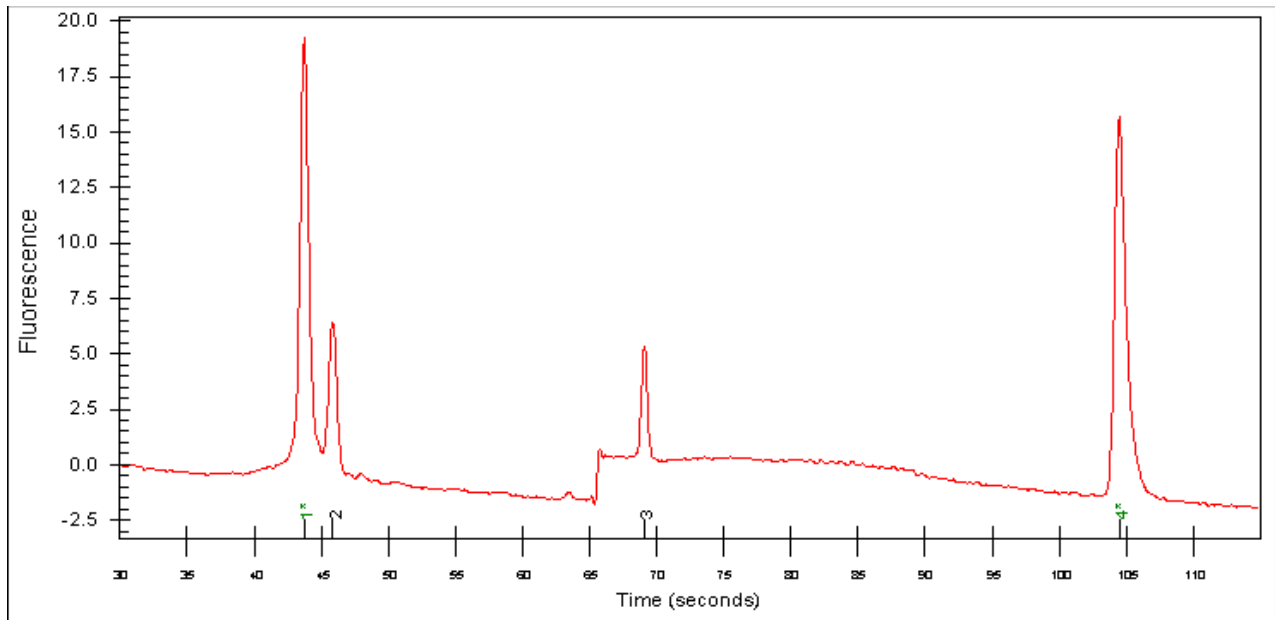
# Poor Baseline: Noise

Most Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.

Least Probable Causes	Solution
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
Autofocus failure.	Check autofocus by means of the <b>Hardware Diagnostics—48</b> . If autofocus fails, call Agilent Technologies.
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> . If the power supply is defective, call Agilent Technologies.

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# Poor Baseline: Jumps



Show me how to solve **Poor Baseline: Jumps**

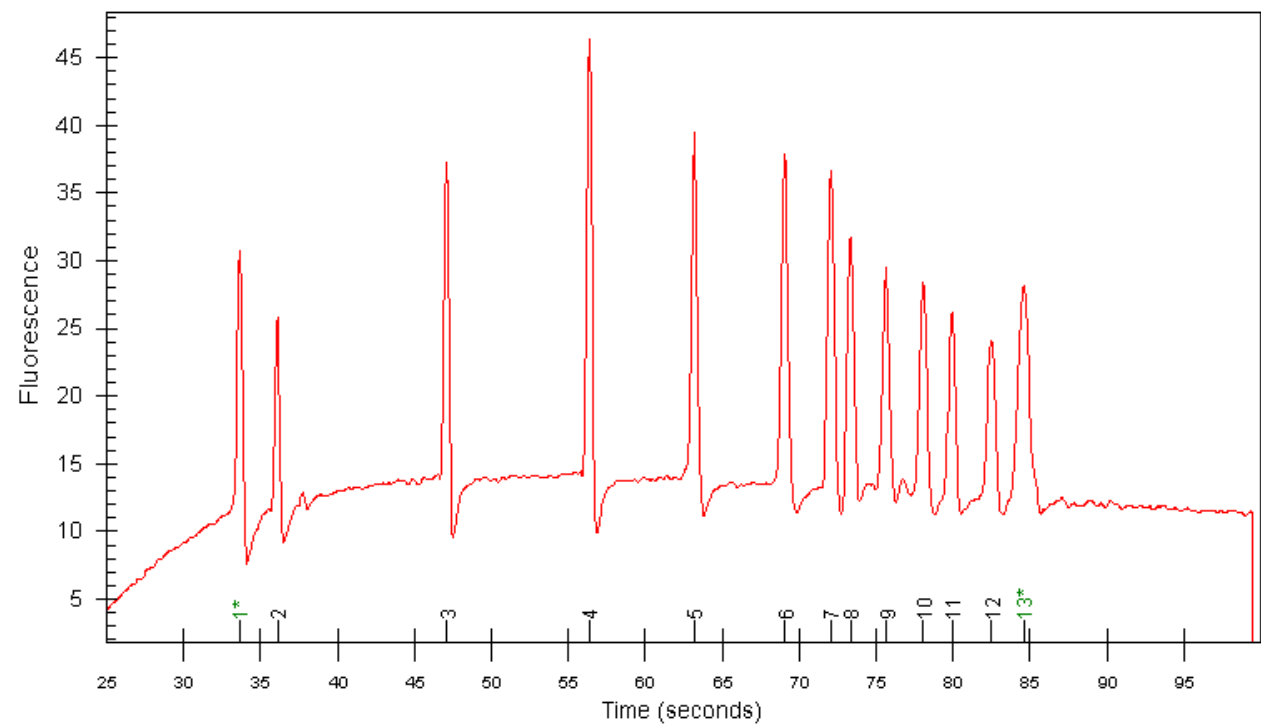
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# Poor Baseline: Jumps

Most Probable Causes	Solution
Vibration of Agilent 2100 Bioanalyzer.	Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Instrument lid was touched during the run.	Don't touch Agilent 2100 Bioanalyzer during a run.
Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Least Probable Causes	Solution
Laser defective.	Check Laser by using the <b>Hardware Diagnostics—48</b> .  If the laser is defective, call Agilent Technologies.
Autofocus failure.	Check autofocus by means of the <b>Hardware Diagnostics—48</b> .  If autofocus fails, call Agilent Technologies.

# Poor Baseline: Bend



Show me how to solve **Poor Baseline: Bend**

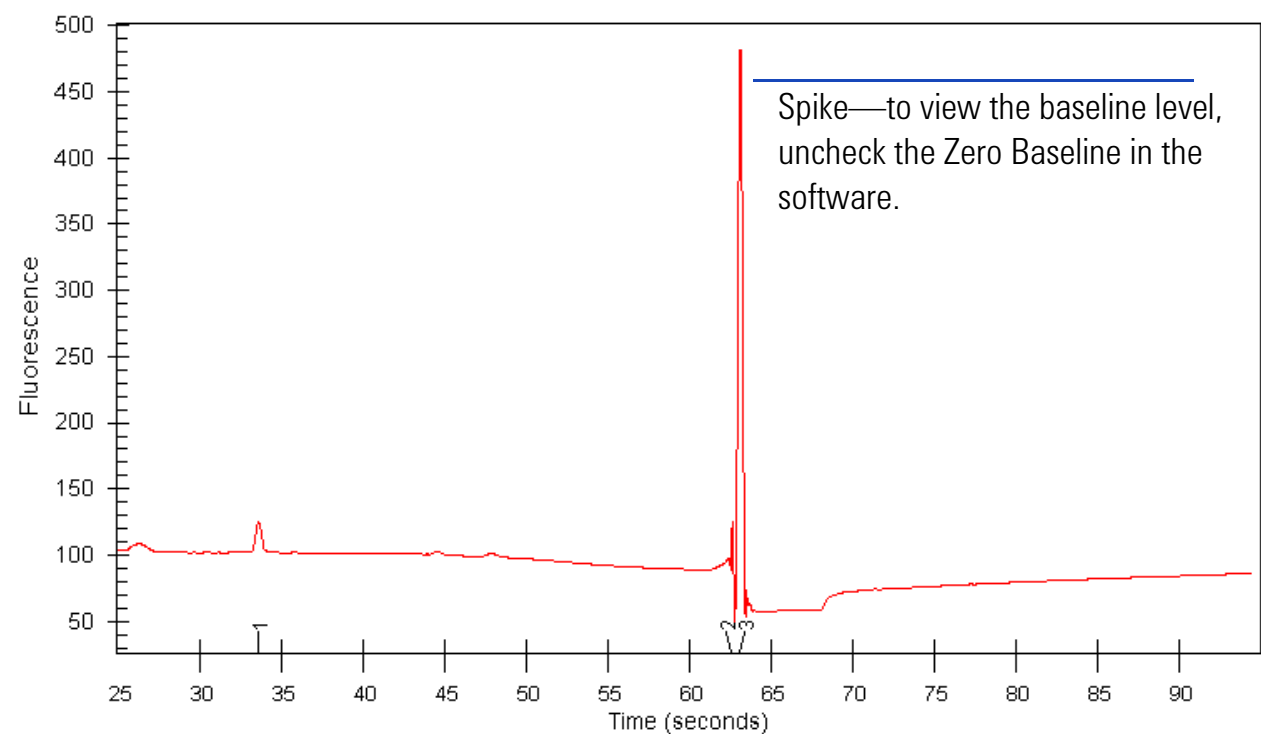
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# Poor Baseline: Bend

Most Probable Causes	Solution
Chip preparation was done with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature before use.  Apply baseline correction algorithm. (software revision A.01.20 or higher).
Chips were stored in the fridge/freezer.	Prepare a new chip. Store chips at room temperature.

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# No Peaks and High Background



Show me how to solve **No Peaks and High Background**

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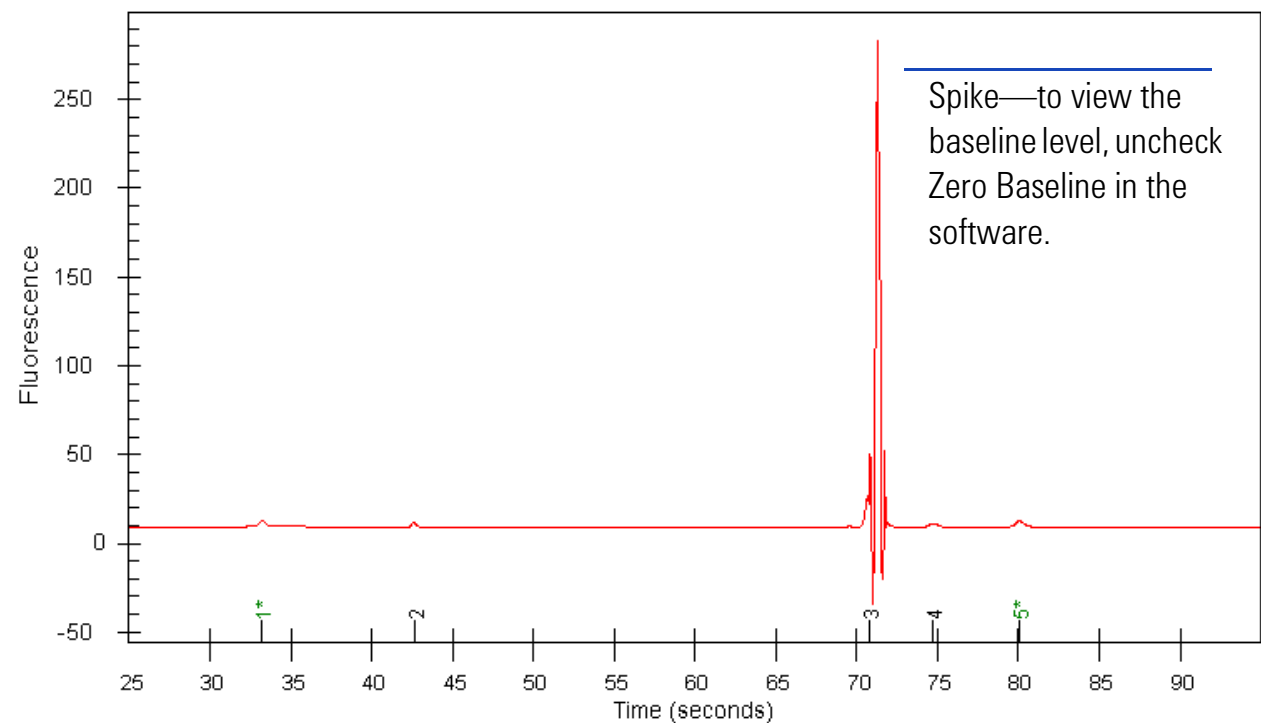
# No Peaks and High Background

Most Probable Causes	Solution
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b>  Replace electrode cartridge.
Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Dye concentration too low (marker disappears).	Use dye concentration according to the DNA Reagent Kit Guide. Let the dye warm up to room temperature before preparing the gel-dye mix.

Least Probable Causes	Solution
Fingerprint on focusing lens.	Clean lens like described in <b>Lens Maintenance—270</b> .
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> . If the power supply is defective, call Agilent Technologies.
Autofocus failure.	Check autofocus using the <b>Hardware Diagnostics—48</b> . If autofocus fails, call Agilent Technologies.

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# No Peaks and Low Background



Show me how to solve No Peaks and Low Background

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# No Peaks and Low Background

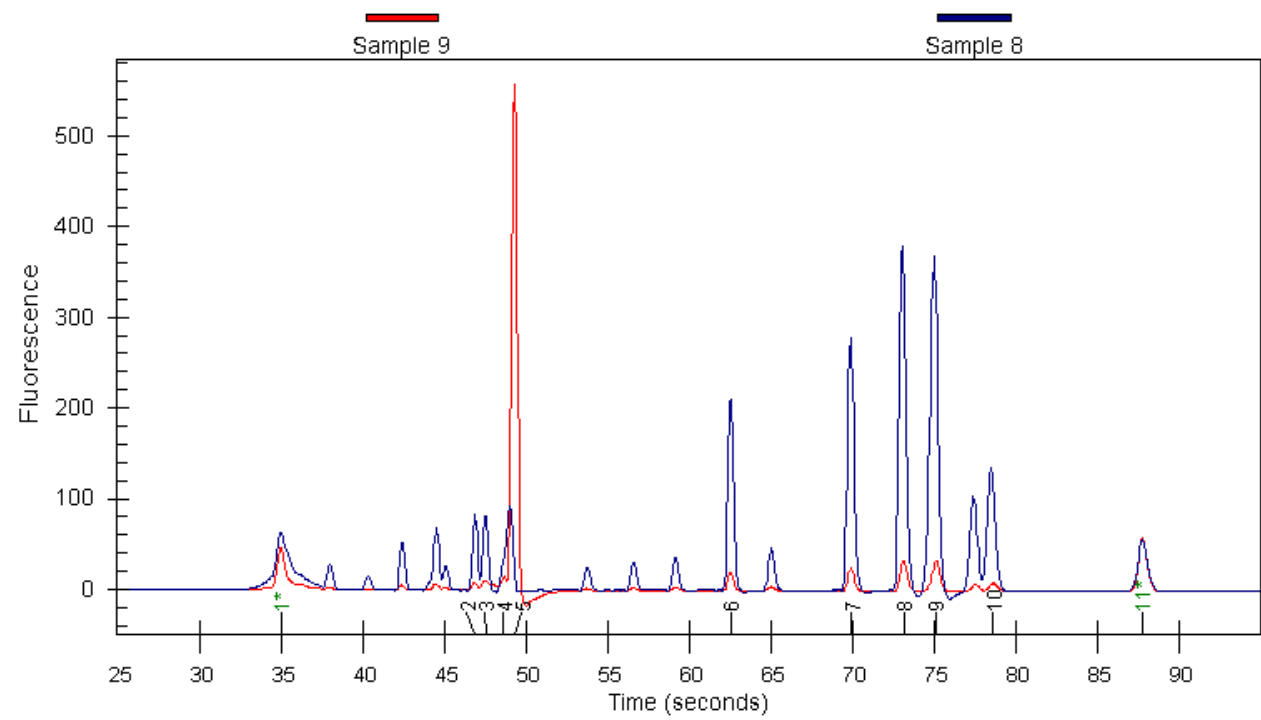
Most Probable Causes	Solution
Dye concentration too low (marker disappears).	Use dye concentration according to the DNA Reagent Kit Guide. Let the dye warm up to room temperature before preparing the gel-dye mix.
Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).



Least Probable Causes	Solution
High voltage power supply defective.	<p>Check high voltage power supply using the <b>Hardware Diagnostics—48</b>.</p> <p>If the power supply is defective, call Agilent Technologies.</p>
Autofocus failure.	<p>Check autofocus using the <b>Hardware Diagnostics—48</b></p> <p>If autofocus fails, call Agilent Technologies.</p>
Laser defective.	<p>Check laser using the <b>Hardware Diagnostics—48</b>.</p> <p>If the laser is defective, call Agilent Technologies.</p>

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# Cross Contamination



Show me how to solve **Cross Contamination**

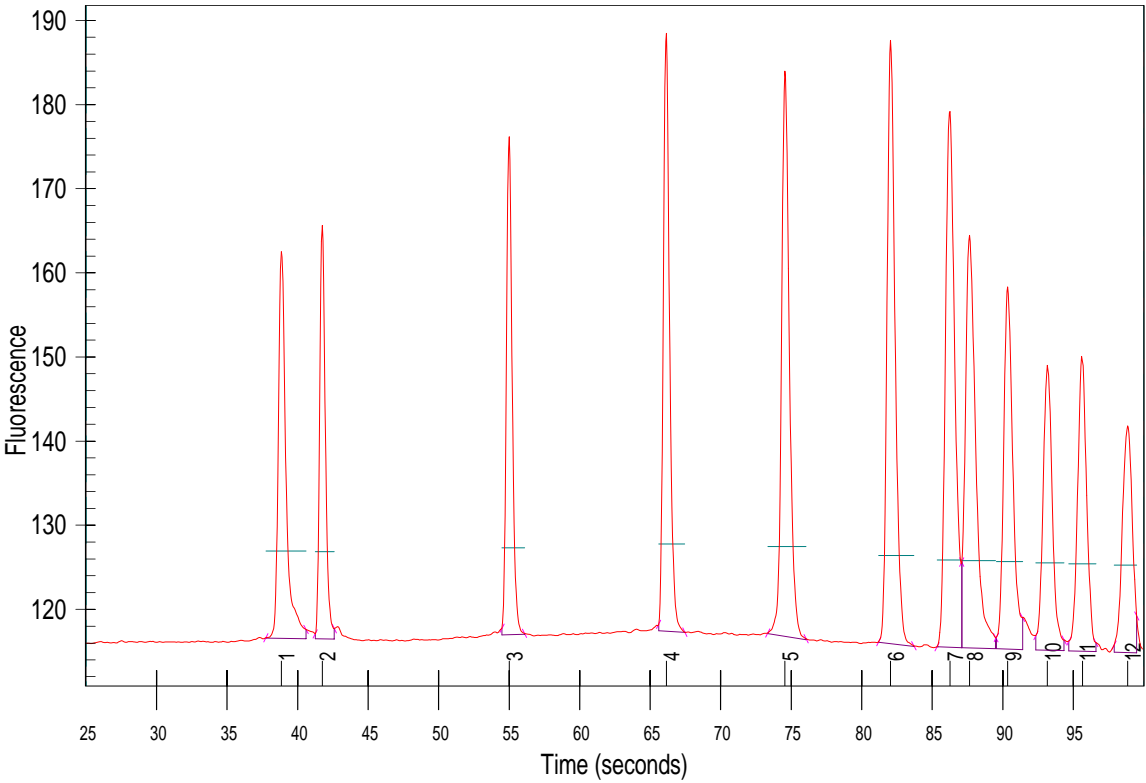
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# Cross Contamination

Most Probable Causes	Solution
Sample concentration too high.	Use sample concentration according to the DNA Reagent Kit Guide.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip and pipette again. Use appropriate pipette and tips.
Probable Causes	Solution
Leak current due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b> . Replace electrode cartridge.

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# Peaks Migrating Late



Show me how to solve Peaks Migrating Late

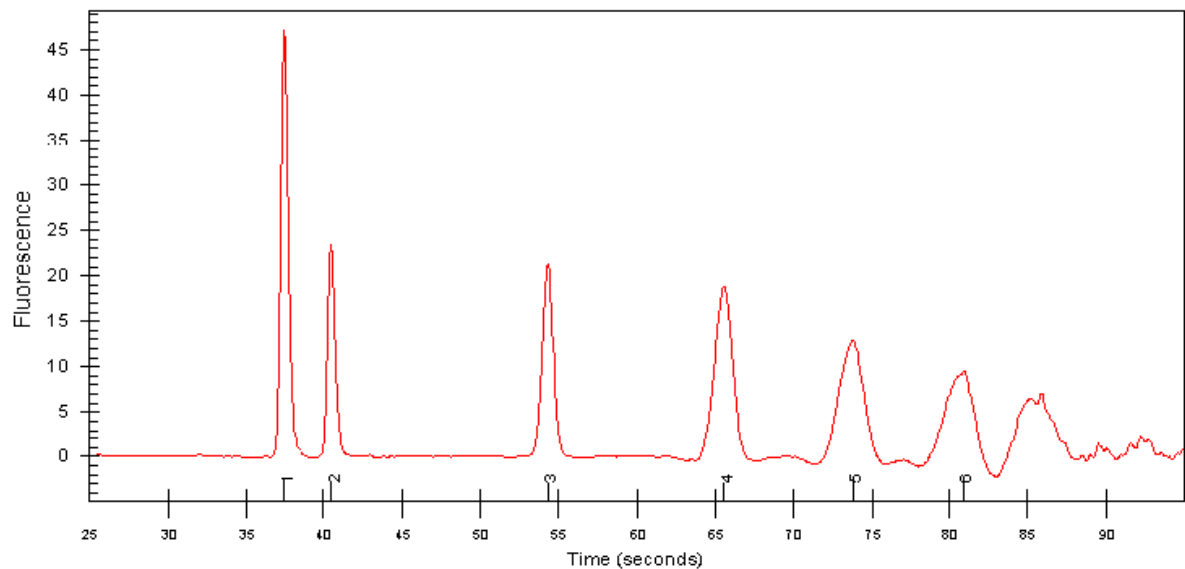
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# Peaks Migrating Late

Most Probable Causes	Solution
Vortex speed too high.	Vortex at lower (medium) speed. For chips use only the IKA vortexer.
Probable Causes	Solution
Vortex adapter not connected tightly.	Press vortex adapter tightly on mount (vortex adapter must not rock).  Replace vortex adapter (p/n 5022-2190) if necessary.

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# Late Migration and Peak Broadening



Show me how to solve **Late Migration and Peak Broadening**

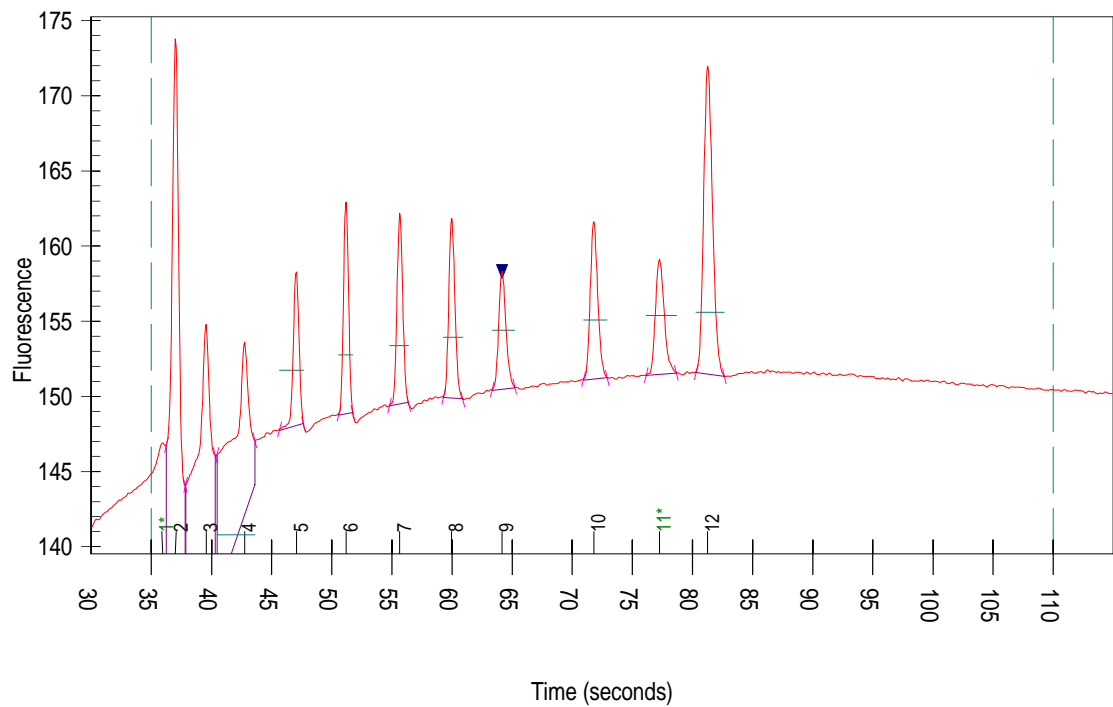
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# Late Migration and Peak Broadening

Most Probable Causes	Solution
Genomic DNA or high molecular weight DNA in following sample well, e.g. uncompletely digested Lambda-DNA.	Perform additional enzymatic digestion with the sample.

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# Bend Ladder Baseline



Show me how to solve **Bend Ladder Baseline**

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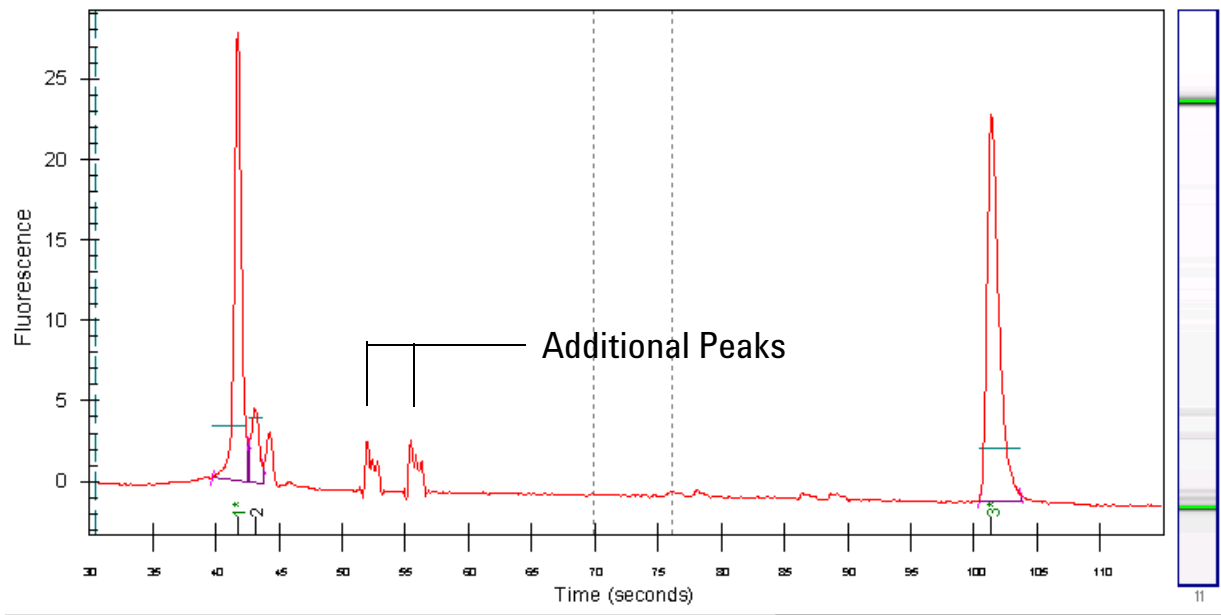
# Bend Ladder Baseline

Most Probable Causes	Solution
Chip preparation was done with cold reagents.	Prepare a new chip.  Allow all reagents and reagent mixes to warm up to room temperature for 30 min before use.  Apply baseline correction algorithm. (software revision A.01.20 or higher).
Chips were stored in the fridge/freezer.	Prepare a new chip.  Store chips at room temperature.

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# List of DNA Electropherograms

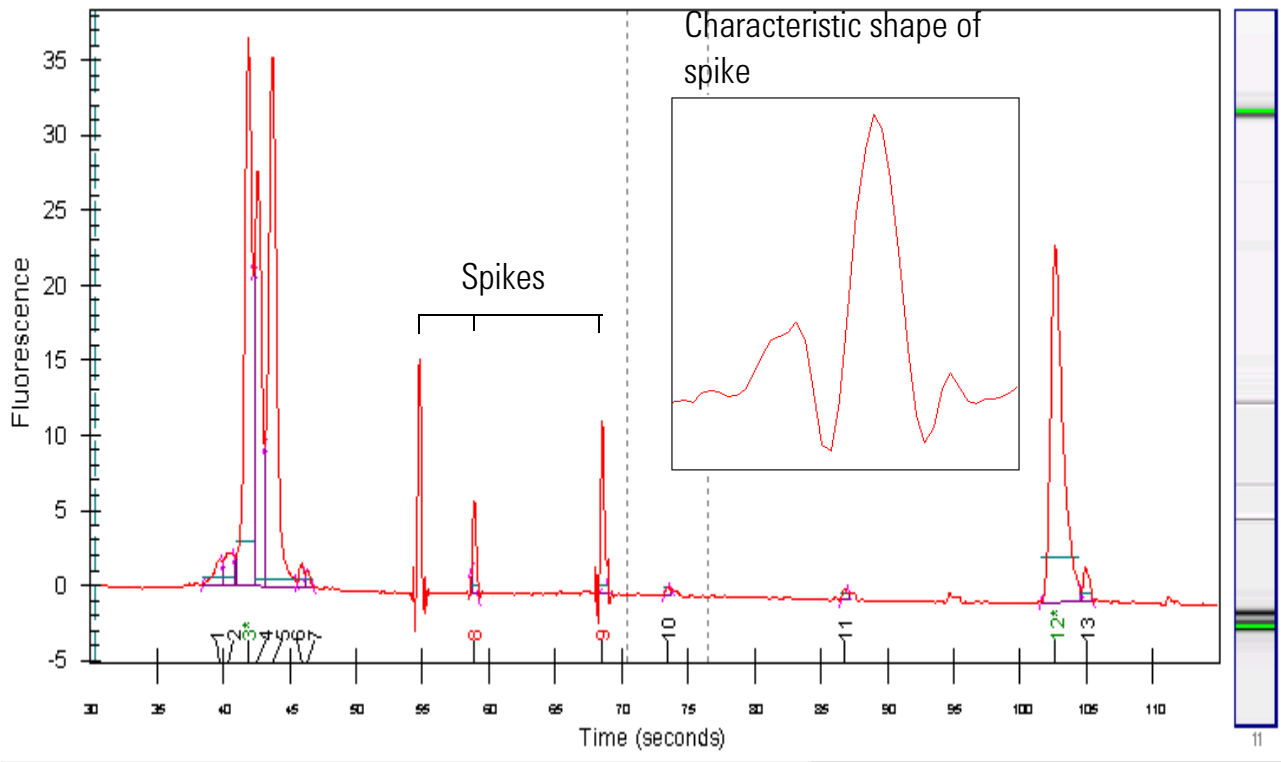
## Additional Sample or Ladder Peaks



Show me how to solve **Additional Sample or Ladder Peaks**

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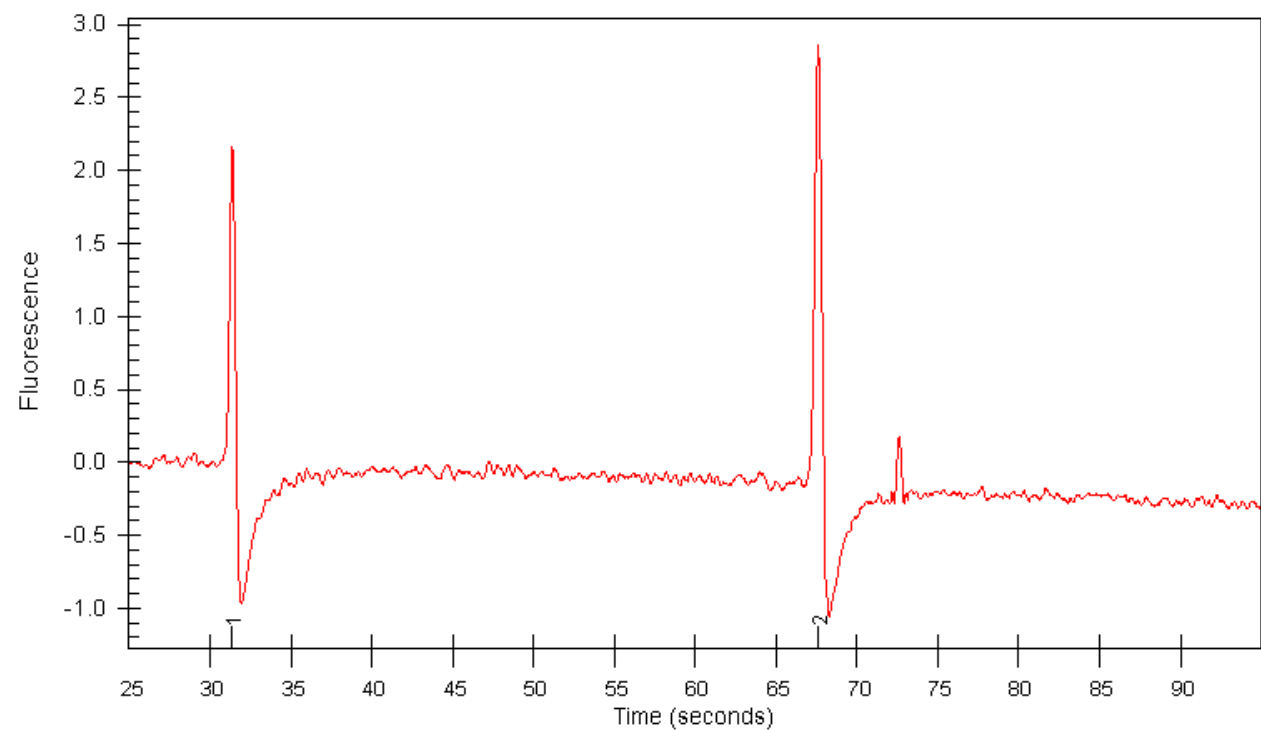
# Spikes/Glitches



Show me how to solve Spikes/Glitches

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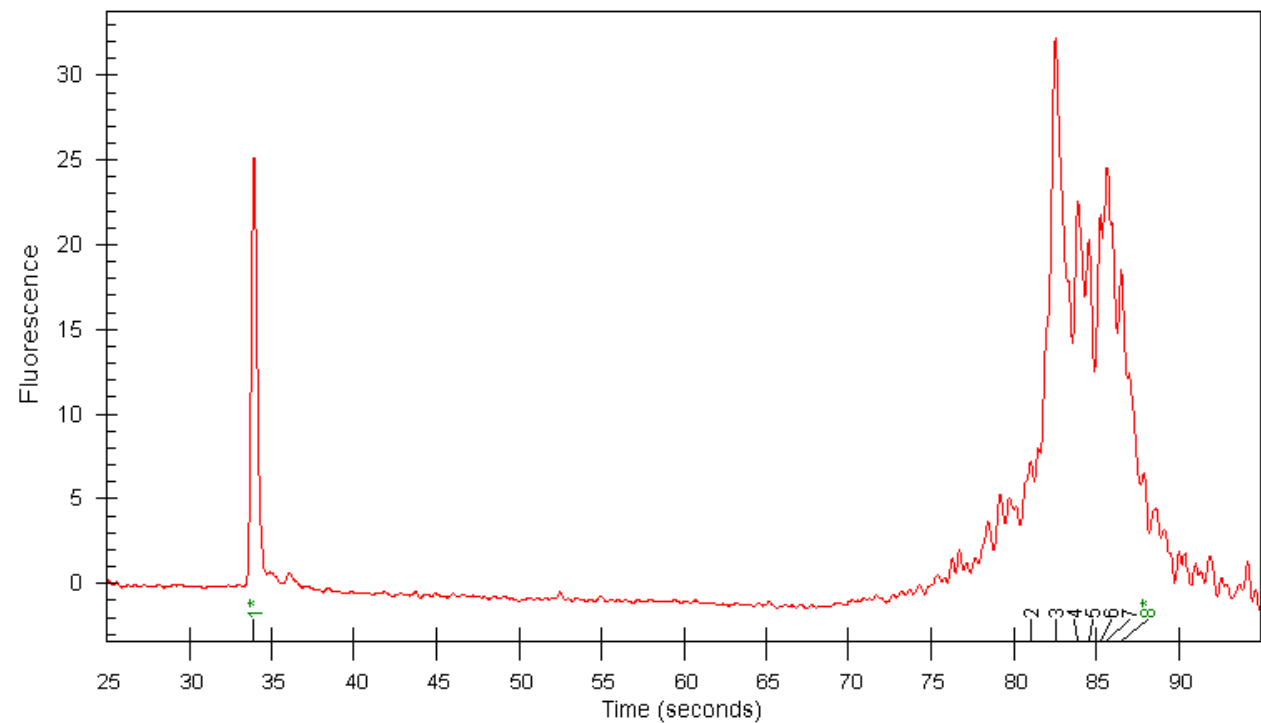
# Poor Sensitivity



Show me how to solve **Poor Sensitivity**

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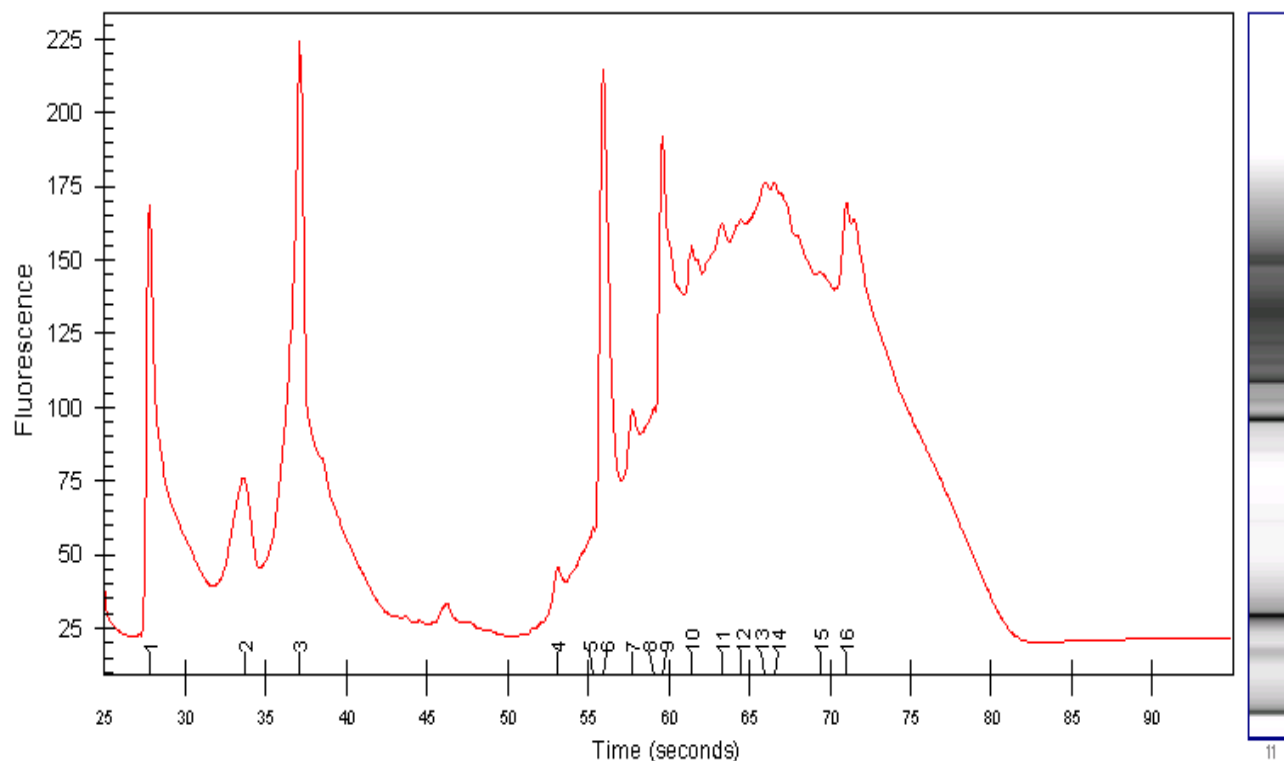
# Noisy Electropherogram



Show me how to solve **Noisy Electropherogram**

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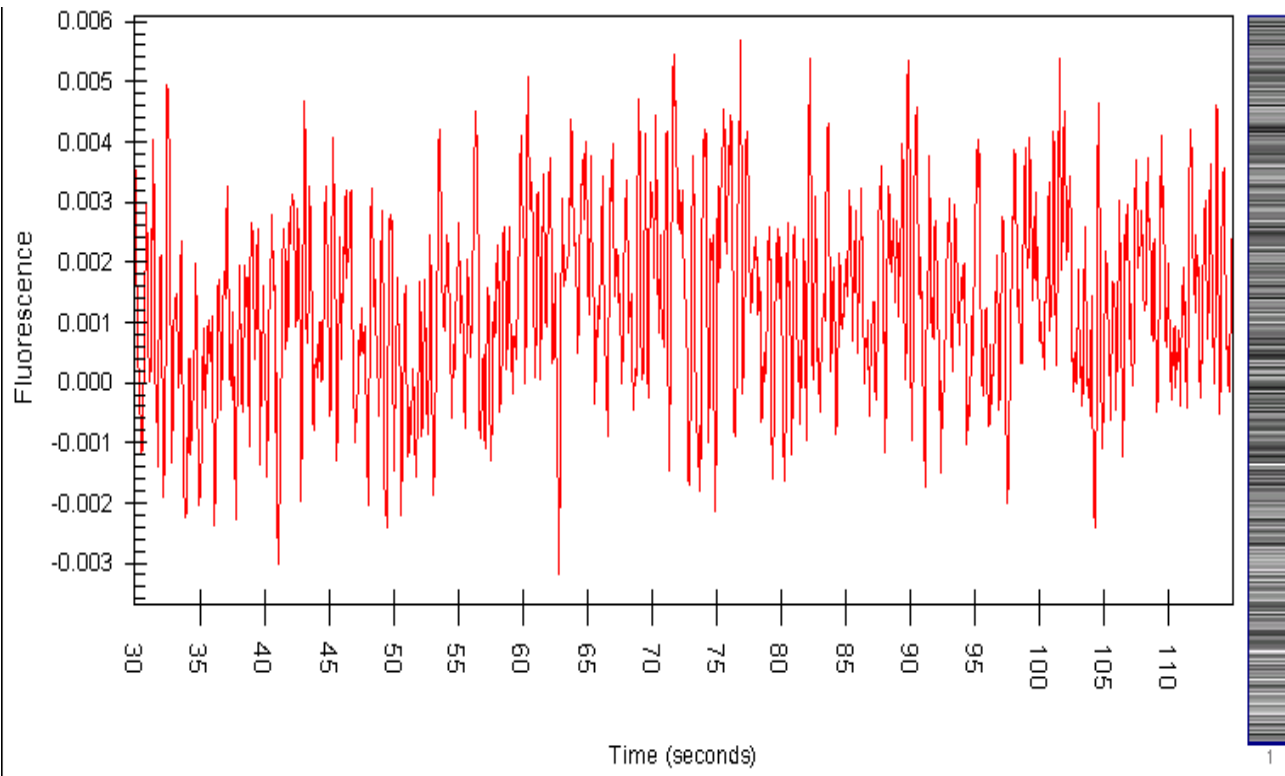
## Broad Peaks



Show me how to solve **Broad Peaks**

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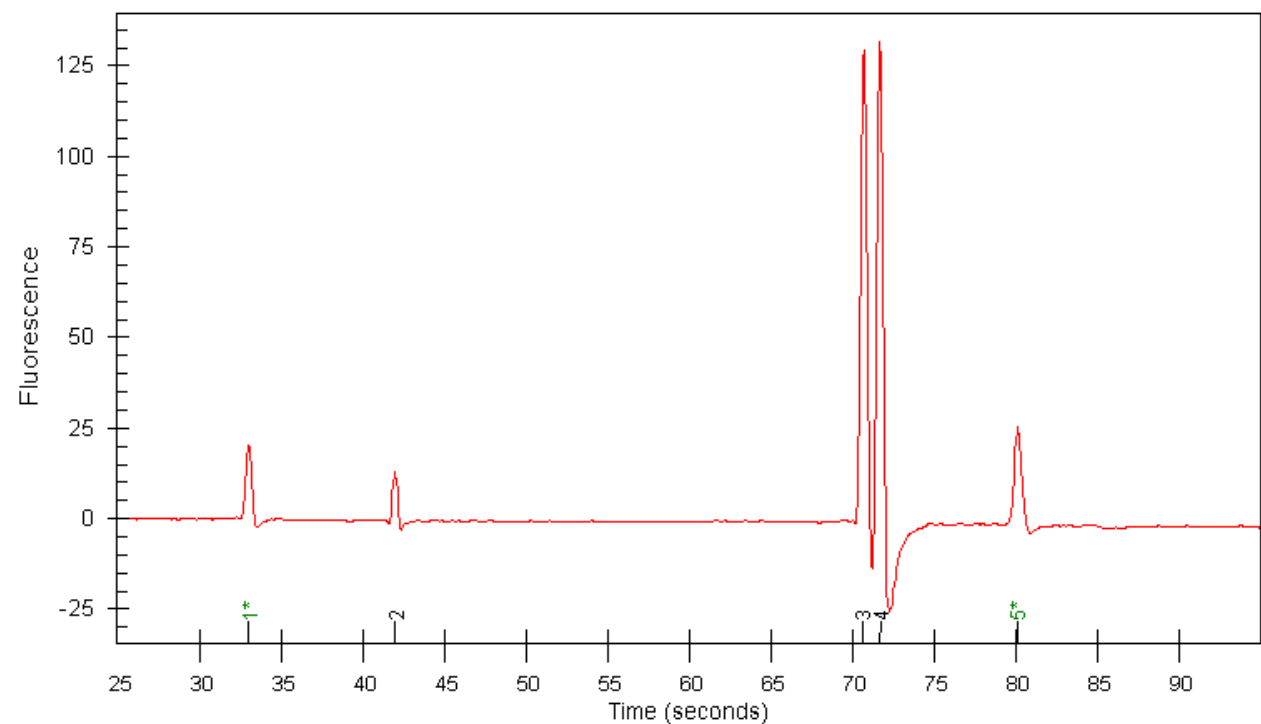
# Missing Peaks or Marker Peaks



Show me how to solve **Missing Peaks or Marker Peaks**

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Poor Baseline: Dips

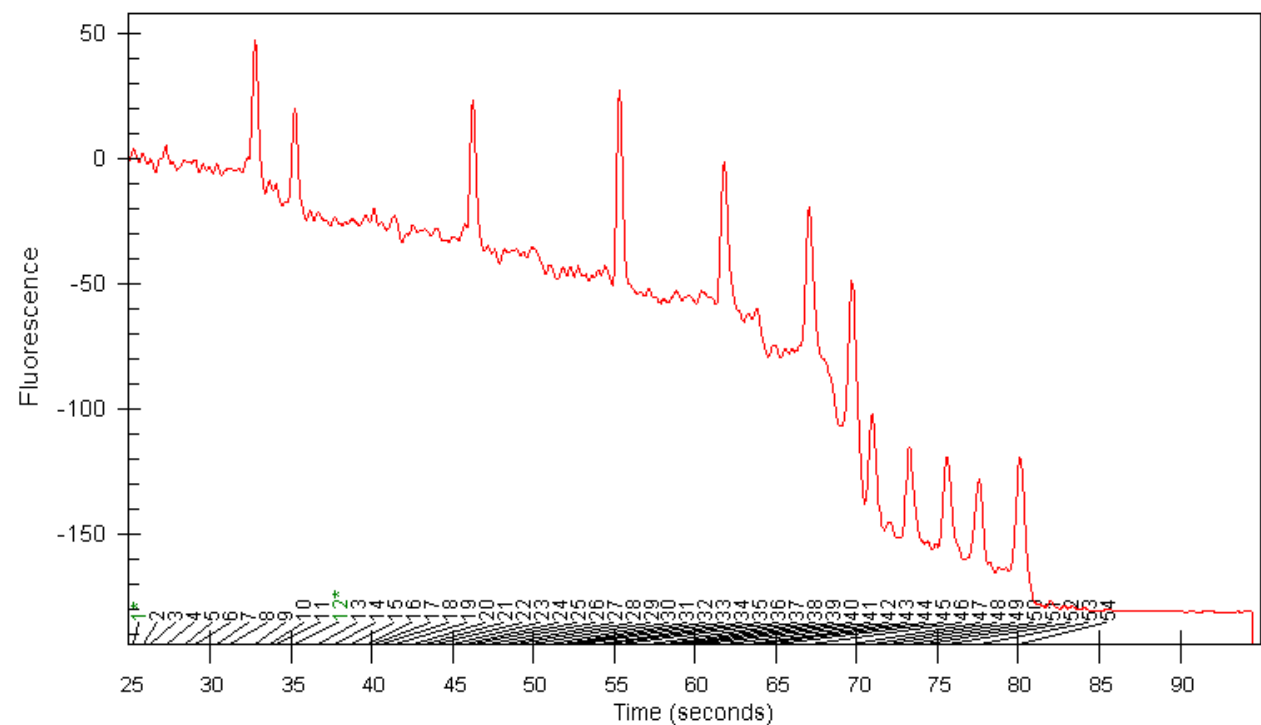


Show me how to solve **Poor Baseline: Dips**

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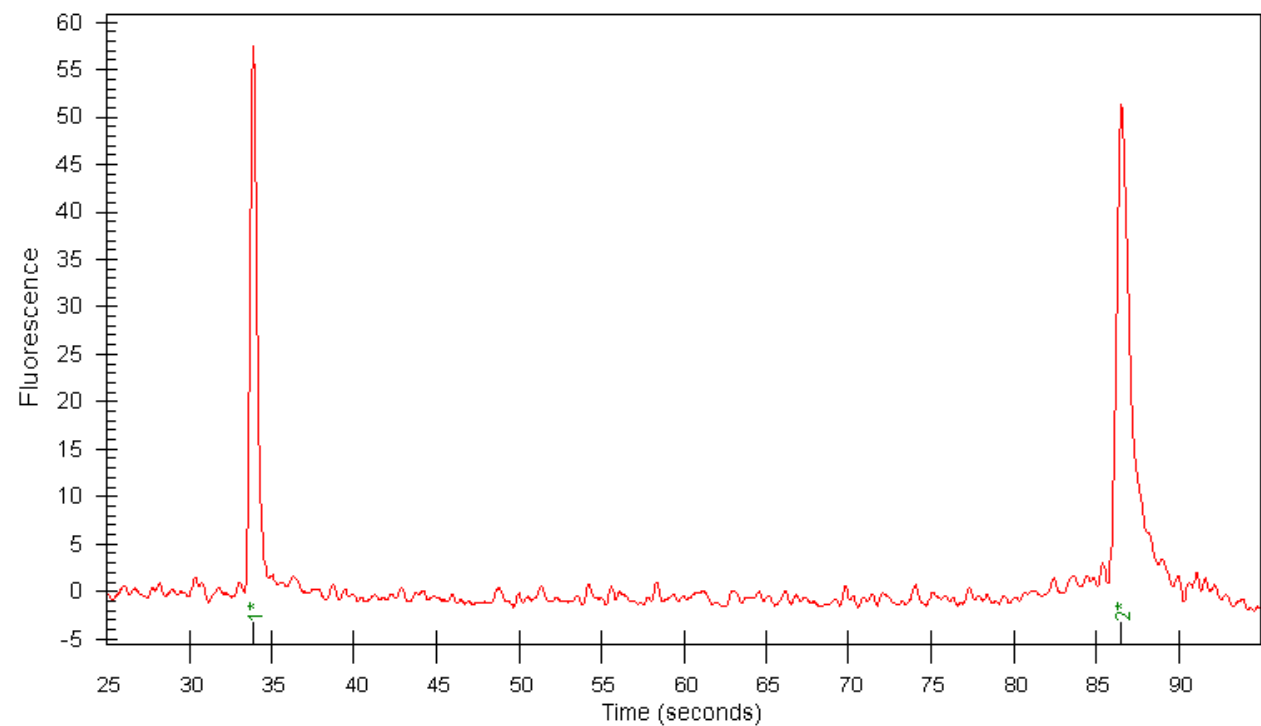
Poor Baseline: Drift



Show me how to solve **Poor Baseline: Drift**

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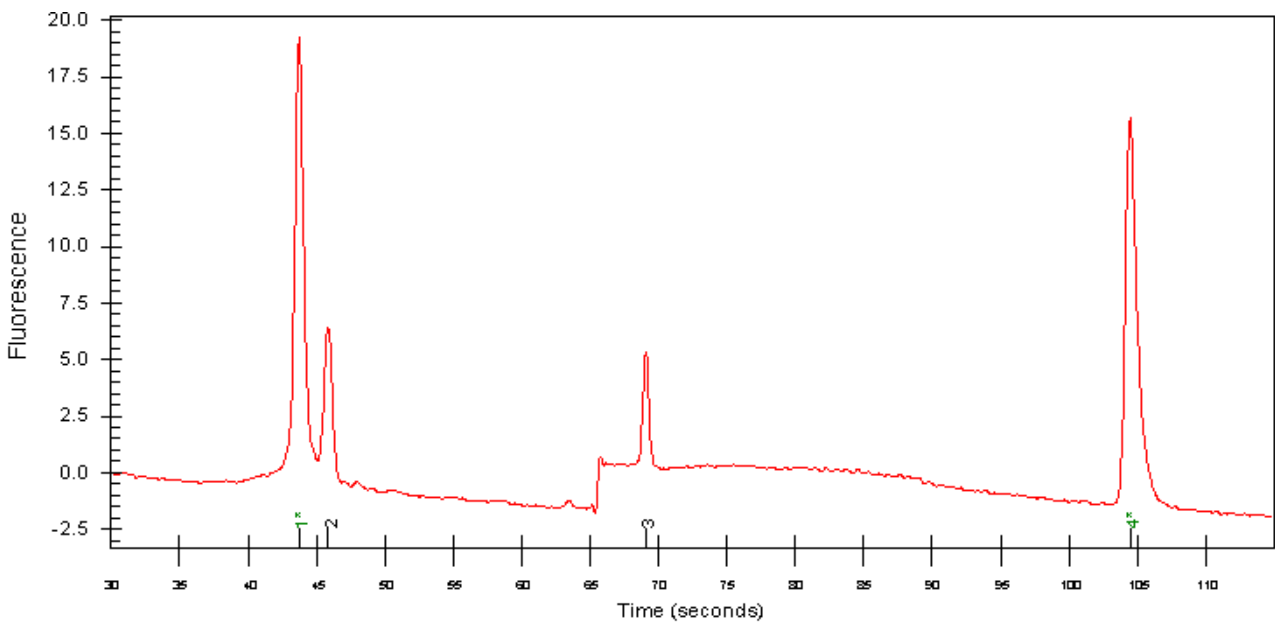
Poor Baseline: Noise



Show me how to solve **Poor Baseline: Noise**

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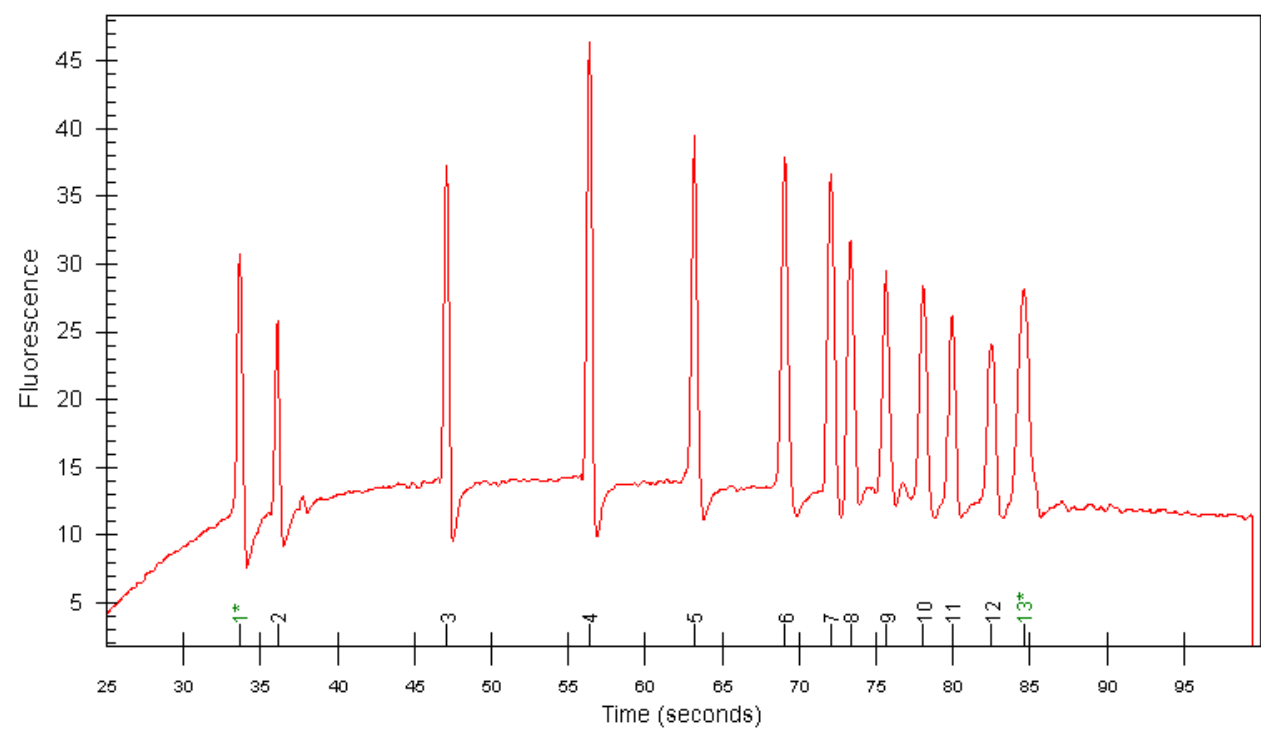
Poor Baseline: Jumps



Show me how to solve **Poor Baseline: Jumps**

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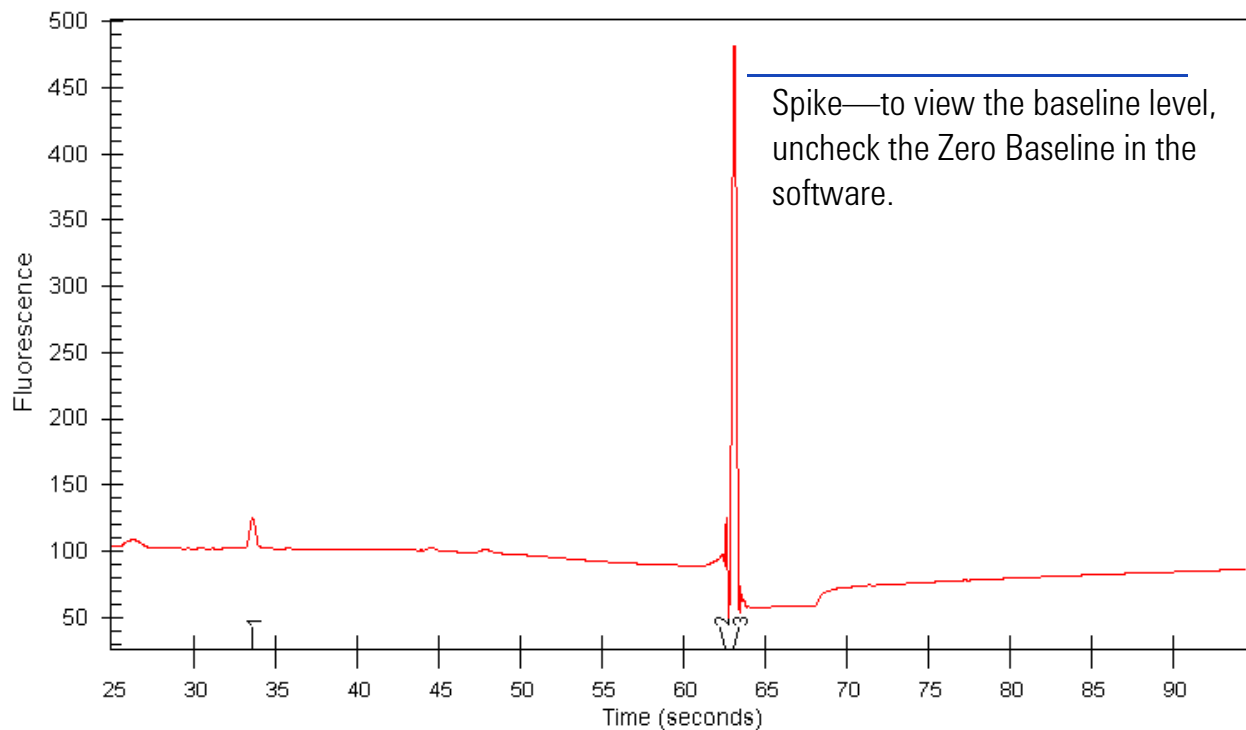
Poor Baseline: Bend



Show me how to solve **Poor Baseline: Bend**

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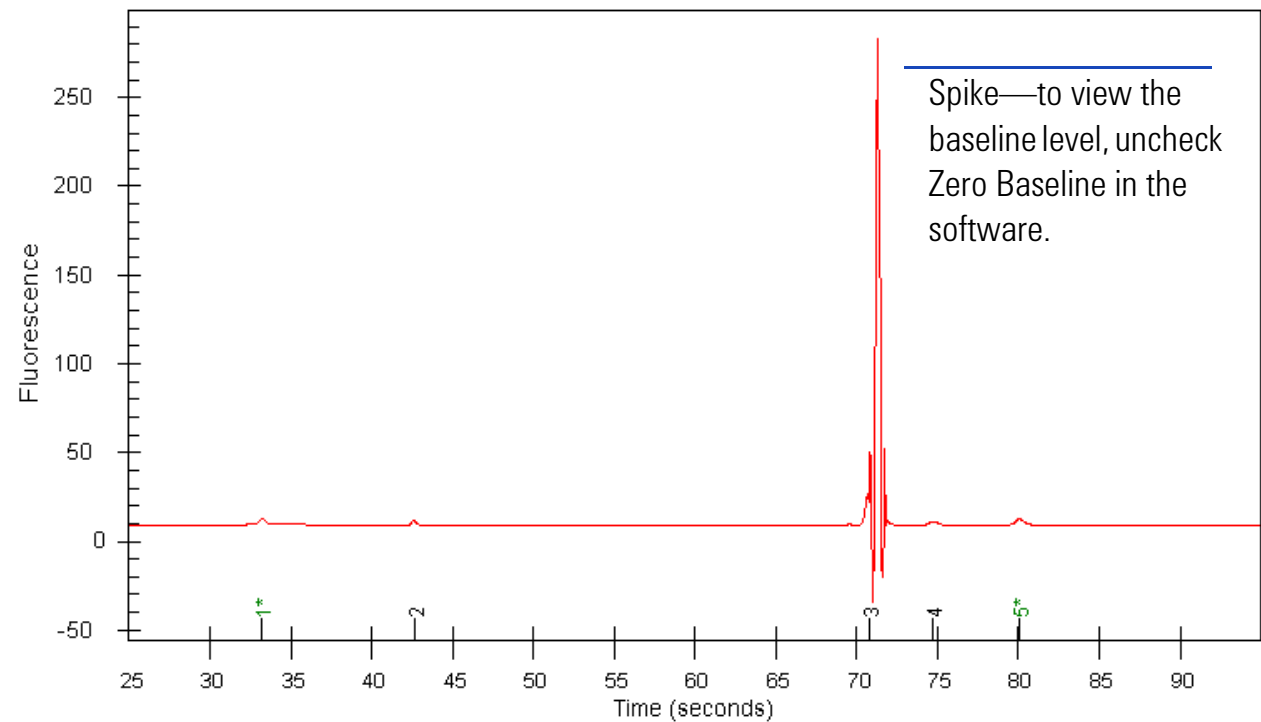
## No Peaks and High Background



Show me how to solve **No Peaks and High Background**

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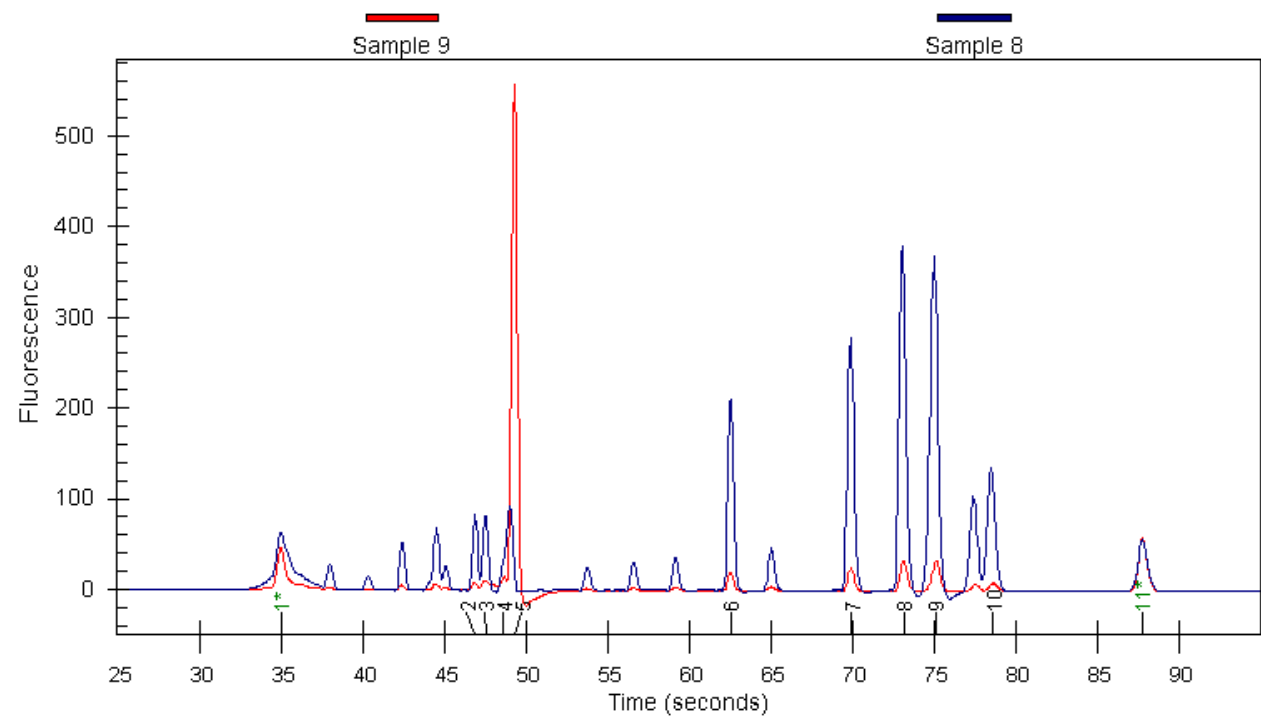
# No Peaks and Low Background



Show me how to solve No Peaks and Low Background

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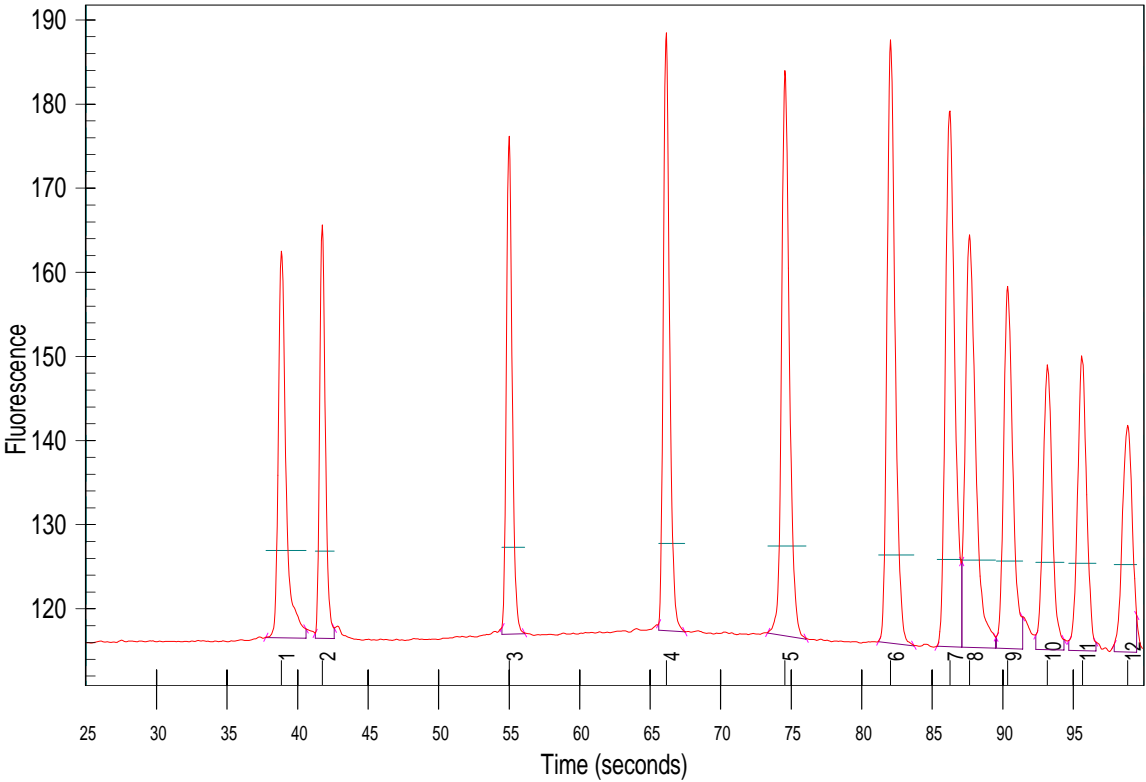
# Cross Contamination



Show me how to solve **Cross Contamination**

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# Peaks Migrating Late

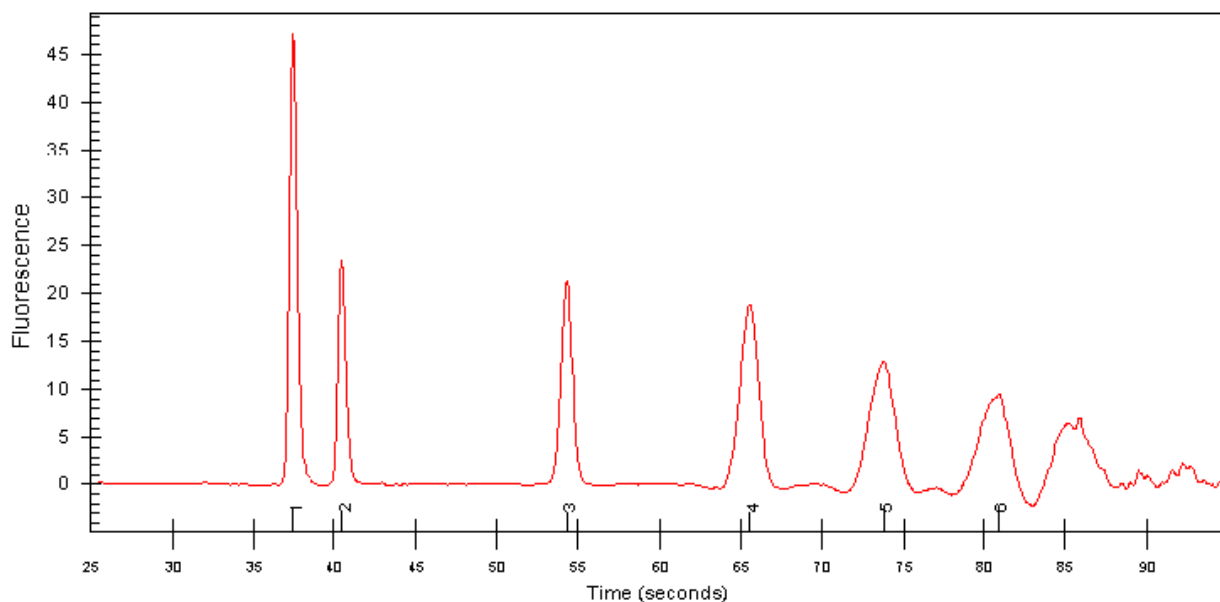


Show me how to solve **Peaks Migrating Late**

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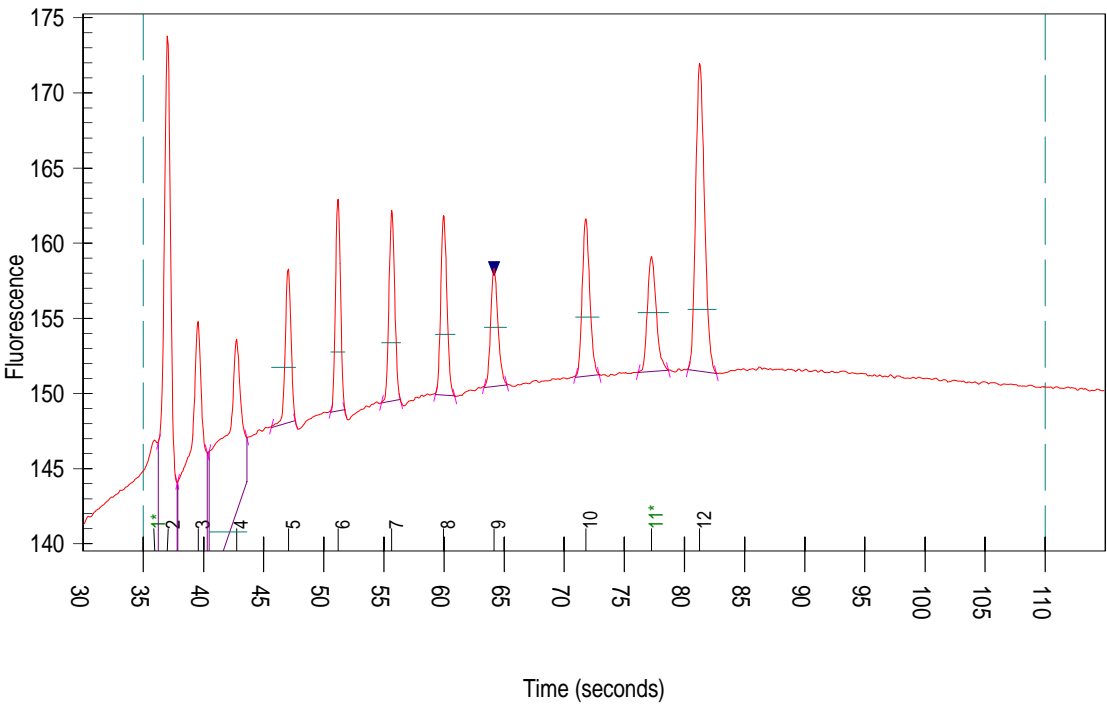
## Late Migration and Peak Broadening



Show me how to solve **Late Migration and Peak Broadening**

[Back to Top of List](#)

# Bend Ladder Baseline



Show me how to solve **Bend Ladder Baseline**

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# Troubleshooting the RNA Application

## Essential Measurement Practices

For hints on how to handle chips and chemicals, see **Essential Measurement Practices—7**.

## Troubleshooting the Application

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Click the  or  button next to the error message to view a help screen that is specific for that error.

Additional information regarding the nature of a problem can often be found in the run log for the data file. Choose Tools > View Log File > Run Log. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by your Agilent 2100 Bioanalyzer might not be what you expected. To help you find the reason for the discrepancy, see **Symptoms—125**.

For most observations you will find at least one corresponding example, depicting a typical electropherogram or result table. Once you have identified the observation that resembles the outcome of your experiment, you will get a set of assigned causes listed by priority.

The causes are grouped into three levels:

- **most probable cause**
- **probable cause**
- **least probable cause**

A list of solutions that help you to fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by priority.

If you are not able to assign a symptom to your problem, compare your electropherogram with the **List of RNA Electropherograms—169**.

# Symptoms

Click the icon to see an example, or go straight to the troubleshooting hints.

Too High Quantification Results—127

Too Low Quantification Results—128

Wrong Quantification Result—130

Chip Not Detected—133

Poor Chip Performance—134



Additional Sample or Ladder Peaks—135



Genomic DNA Contamination—138



Degraded RNA Ladder and/or Samples—140



Spikes/Glitches—142



Poor Sensitivity—145



Noisy Electropherogram—148



Broad Peaks—151



Missing Peaks—154



Poor Baseline: Dips—157



Poor Baseline: Drift—159



Noisy Electropherogram—148



Wavy Baseline—162



Cross Contamination—165



Late Migration of RNA Ladder or Samples—167

List of RNA Electropherograms—169

# Too High Quantification Results

Most Probable Causes	Solution
Pipetting error during preparation of mixtures.	Check dilution procedure.  Check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid.  Use appropriate pipette and tips.
Probable Causes	Solution
Dye concentration too low.	Use dye concentration according to the RNA Reagent Kit Guide. Let the dye warm up to room temperature before preparing the gel-dye mix.
Least Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.

Back to **Symptoms**

# Too Low Quantification Results

Most Probable Causes	Solution
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use the IKA vortexer. Adjust the speed to the set-point.
Inaccurate reference measurement (e.g. UV-absorption) due to remaining UV absorbing solvent in the sample.	Purify sample for UV measurement.
Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Sample concentration too high.	Use sample concentration according to the RNA Reagent Kit Guide.



Least Probable Causes	Solution
Dye concentration too high.	Use dye concentration according to the RNA Reagent Kit Guide. Let the dye warm up to room temperature before preparing the gel-dye mix.

---

Back to **Symptoms**

# Wrong Quantification Result

Most Probable Causes	Solution
RNA ladder degraded.	Use new ladder aliquot/chip. <b>Always wear gloves when handling chips/RNA samples to prevent them from getting contaminated.</b>  Follow decontamination procedure, see <b>Maintenance—246.</b>
Electrodes contaminated with RNAses.	Clean electrodes with RNaseZAP.  Follow decontamination procedure, see <b>Maintenance—246.</b>
Wrong time window in ribosomal peak assignment selected.	Select correct time window.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see RNA Reagent Kit Guide).

Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
No ladder in ladder well.	Use a new chip.
Probable Causes	Solution
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use the IKA vortexer. Adjust the speed to the set-point.

Least Probable Causes	Solution
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 Bioanalyzer in thermally stable environment.
High voltage power supply defective.	<p>Check high voltage power supply using the <b>Hardware Diagnostics—48</b>.</p> <p>If the power supply is defective, call Agilent Technologies.</p>
Laser defective.	<p>Check laser using the <b>Hardware Diagnostics—48</b>.</p> <p>If the laser is defective, call Agilent Technologies.</p>

Back to **Symptoms**

# Chip Not Detected

Most Probable Causes	Solution
Amount of liquid pipetted is too low or chip is empty.	Check assay procedure on amount of liquid to be pipetted. Pipette sample or buffer in all wells.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see RNA Reagent Kit Guide).
Probable Causes	Solution
No communication between Agilent 2100 Bioanalyzer and PC.	Check whether serial cable is connected.  Check status control image of Agilent 2100 Bioanalyzer (open and close the lid.)
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> If the power supply is defective, call Agilent Technologies.

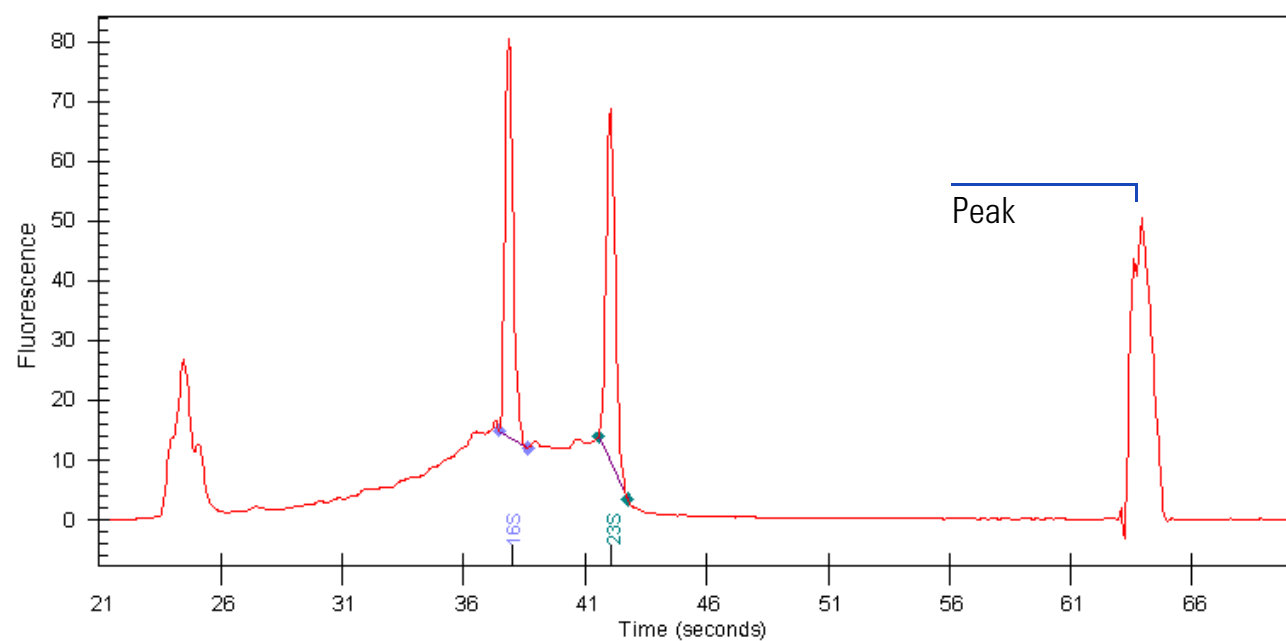
Back to **Symptoms**

# Poor Chip Performance

Most Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see RNA Reagent Kit Guide).
Probable Causes	Solution
Chip preparation was done with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature before use.
Chips were stored in the fridge/freezer.	Prepare a new chip. Store chips at room temperature.
Amount of liquid pipetted is too low or chip is empty.	Check assay procedure on amount of liquid to be pipetted.
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> . If the power supply is defective, call Agilent Technologies.

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# Additional Sample or Ladder Peaks



Show me how to solve Additional Sample or Ladder Peaks

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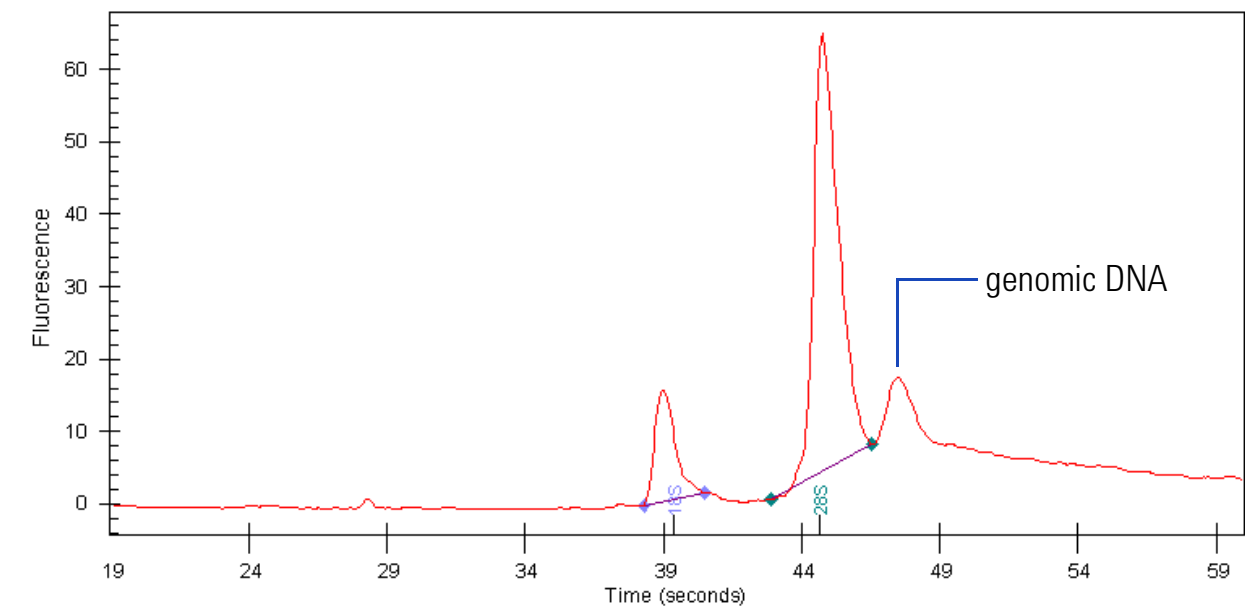
# Additional Sample or Ladder Peaks

Most Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only.
Dust particles in separation channels.	Don't touch the wells of the chip.  Clean the electrodes.  Load the chip immediately after taking it out of its sealed bag.
Particles of protective foam pad on electrode cartridge.	Make sure to remove foam particles of the electrode cartridge before use.
Contamination with Genomic DNA.	Refer to <b>Genomic DNA Contamination—139</b>



Probable Causes	Solution
RNA ladder/sample not denaturated properly.	Heat ladder/samples at 70°C for 2 min.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see RNA Reagent Kit Guide).
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Too high salt concentration in sample.	Use salt concentration according to the RNA Reagent Kit Guide.
Least Probable Causes	Solution
RNA ladder degraded.	Use new ladder aliquot/chip. <b>Always wear gloves when handling chips/RNA samples to prevent them from getting contaminated.</b>  Follow decontamination procedure, see <b>Maintenance—246</b> .

# Genomic DNA Contamination



Show me how to solve **Genomic DNA Contamination**

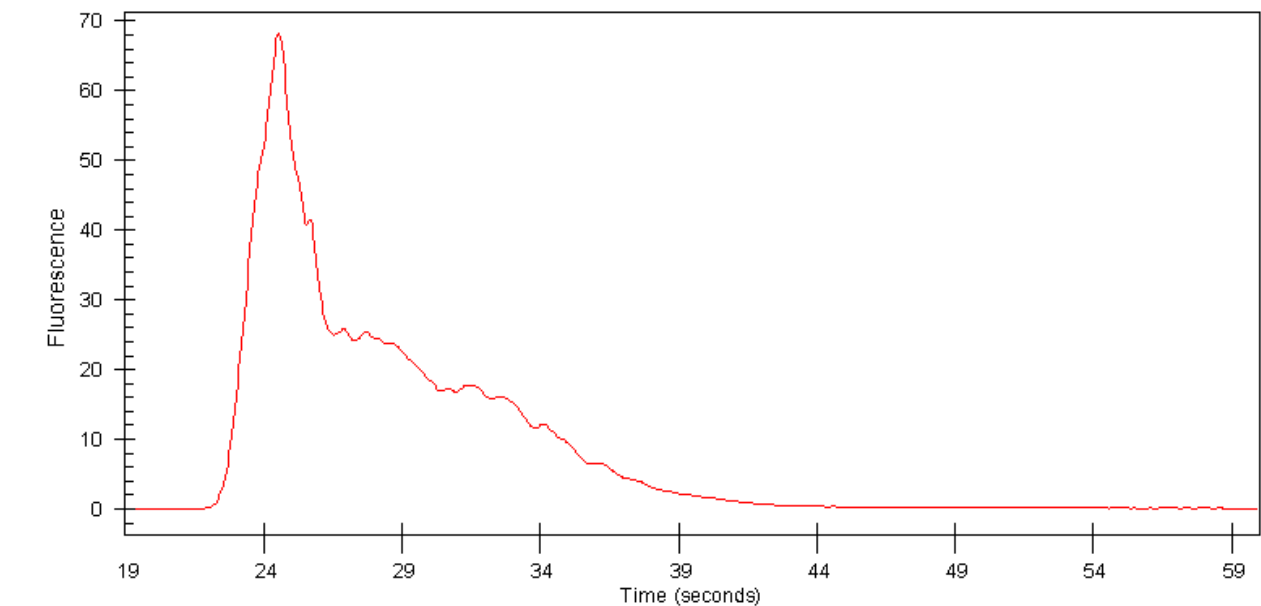
Back to **Symptoms**

# Genomic DNA Contamination

Most Probable Causes	Solution
RNA Isolation Protocol.	Check RNA-isolation protocol.  To remove genomic DNA, perform DNase treatment.

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# Degraded RNA Ladder and/or Samples



Show me how to solve **Degraded RNA Ladder and/or Samples**

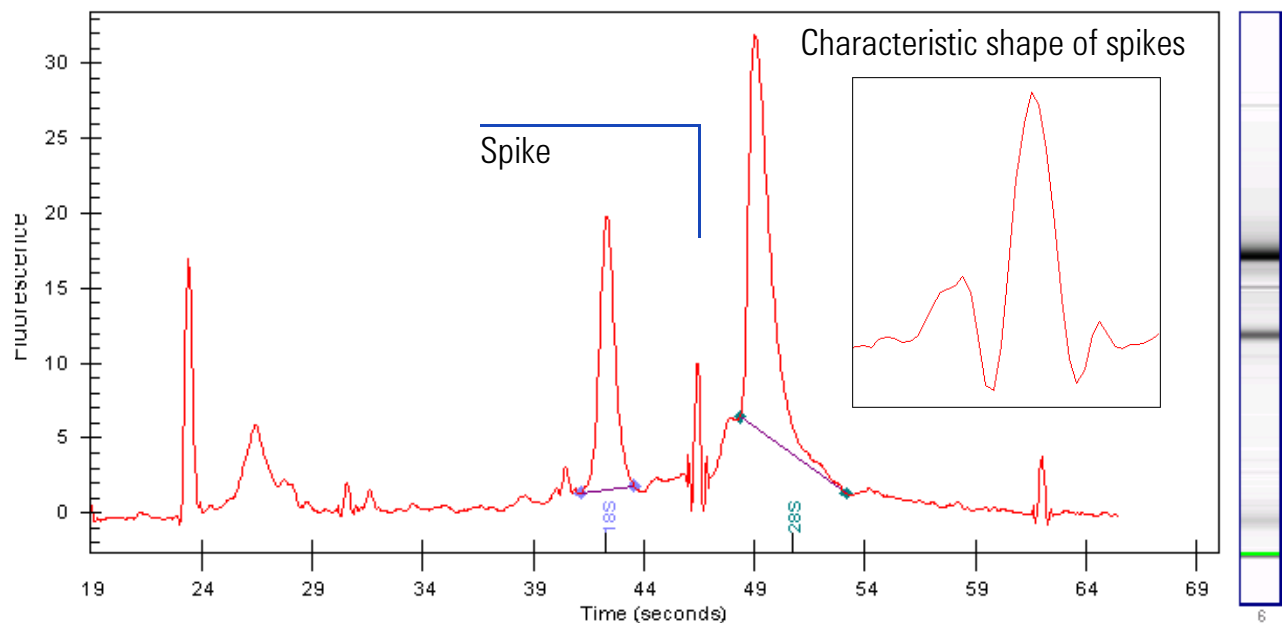
Back to **Symptoms**

# Degraded RNA Ladder and/or Samples

Most Probable Causes	Solution
RNAse contamination of the cartridge.	Clean the electrode cartridge.  Refer to <b>Maintenance—246</b> for details.
RNAse contamination of chips and/or reagents.	Use a new chip and/or fresh reagents. Wear powder-free gloves when preparing the chip.

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# Spikes/Glitches



Show me how to solve **Spikes/Glitches**

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# Spikes/Glitches

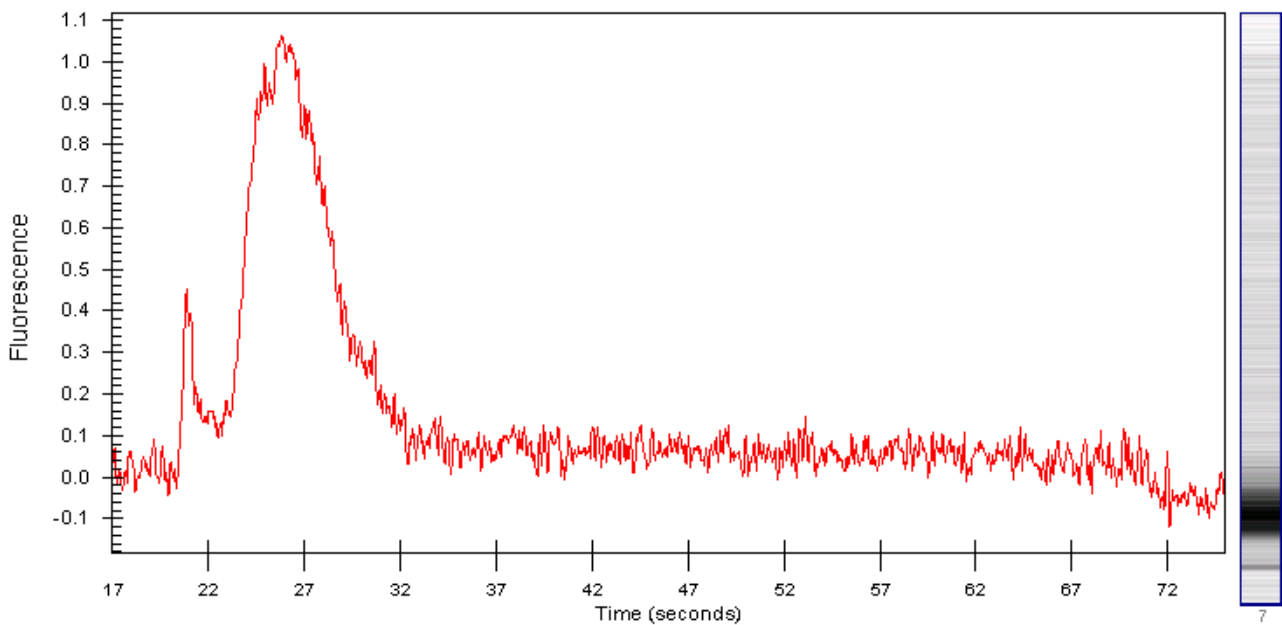
Most Probable Causes	Solution
Chip/gel-dye mix contaminated.	Prepare new chip with new gel-dye mix:  Wear powder-free gloves only.  Don't touch the underside of the chip.  Don't touch the wells of the chip.  Clean the electrodes.  Load the chip immediately after taking it out of its sealed bag.
Gel-dye mix not properly prepared.	Refer to the Reagent Kit Guide for proper preparation of the gel-dye mix. Let the dye warm up to room temperature before preparing the gel-dye mix.
Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see RNA Reagent Kit Guide).

Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run.  Remove vibration devices, such as vortexers and vacuum pumps, from bench.
RNA ladder/sample not denaturated properly.	Heat ladder/samples at 70°C for 2 min.
Least Probable Causes	Solution
Power outlett	Install power filter.

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# Poor Sensitivity



Show me how to solve **Poor Sensitivity**

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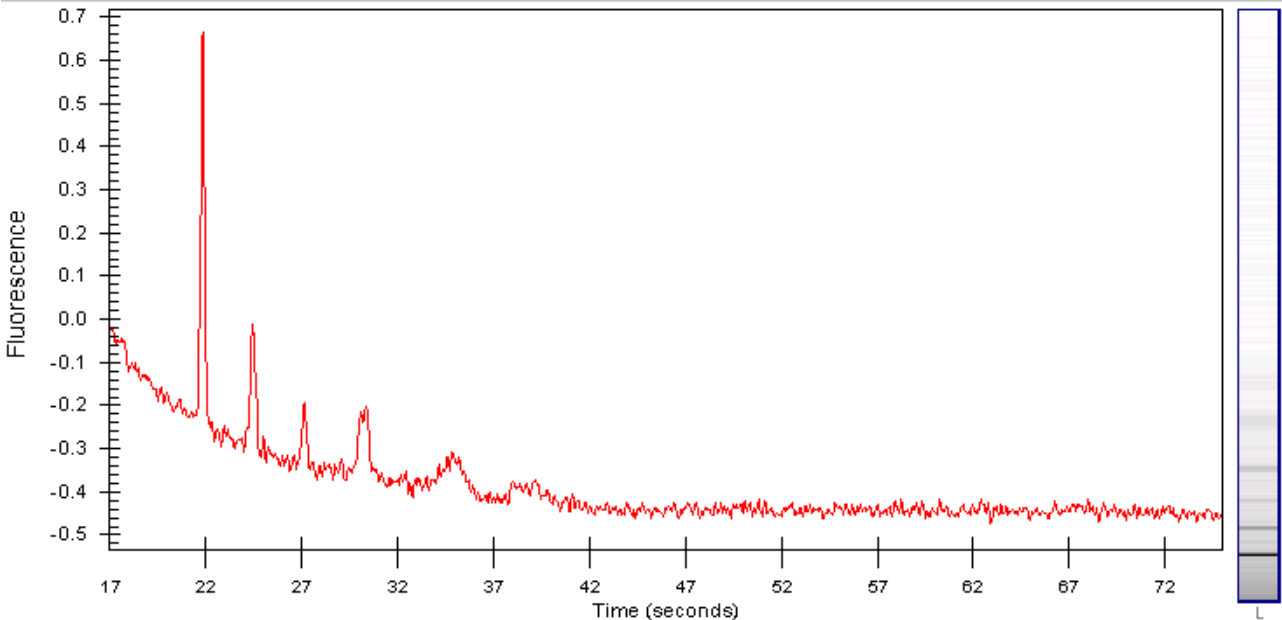
# Poor Sensitivity

Most Probable Causes	Solution
Dye concentration too low.	Use dye concentration according to the Reagent Kit Guide.  Let the dye warm up to room temperature before preparing the gel-dye mix.
Pipetting error during preparation of mixtures.	Check dilution procedure.  Check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid.  Use appropriate pipette and tips.
Probable Causes	Solution
Fingerprint on focusing lens.	Clean lens like described in <b>Lens Maintenance—270</b> .
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use IKA shaker for chip vortexing. Adjust speed to set-point.

Least Probable Causes	Solution
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
Vibration of Agilent 2100 Bioanalyzer.	<p>Don't touch Agilent 2100 Bioanalyzer during a run.</p> <p>Remove vibration devices, such as vortexers and vacuum pumps, from bench.</p>
Autofocus failure.	Check autofocus using the <b>Hardware Diagnostics—48</b> . If autofocus fails, call Agilent Technologies.
Laser defective.	Check laser using the <b>Hardware Diagnostics—48</b> . If the laser is defective, call Agilent Technologies.

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# Noisy Electropherogram



Show me how to solve Noisy Electropherogram

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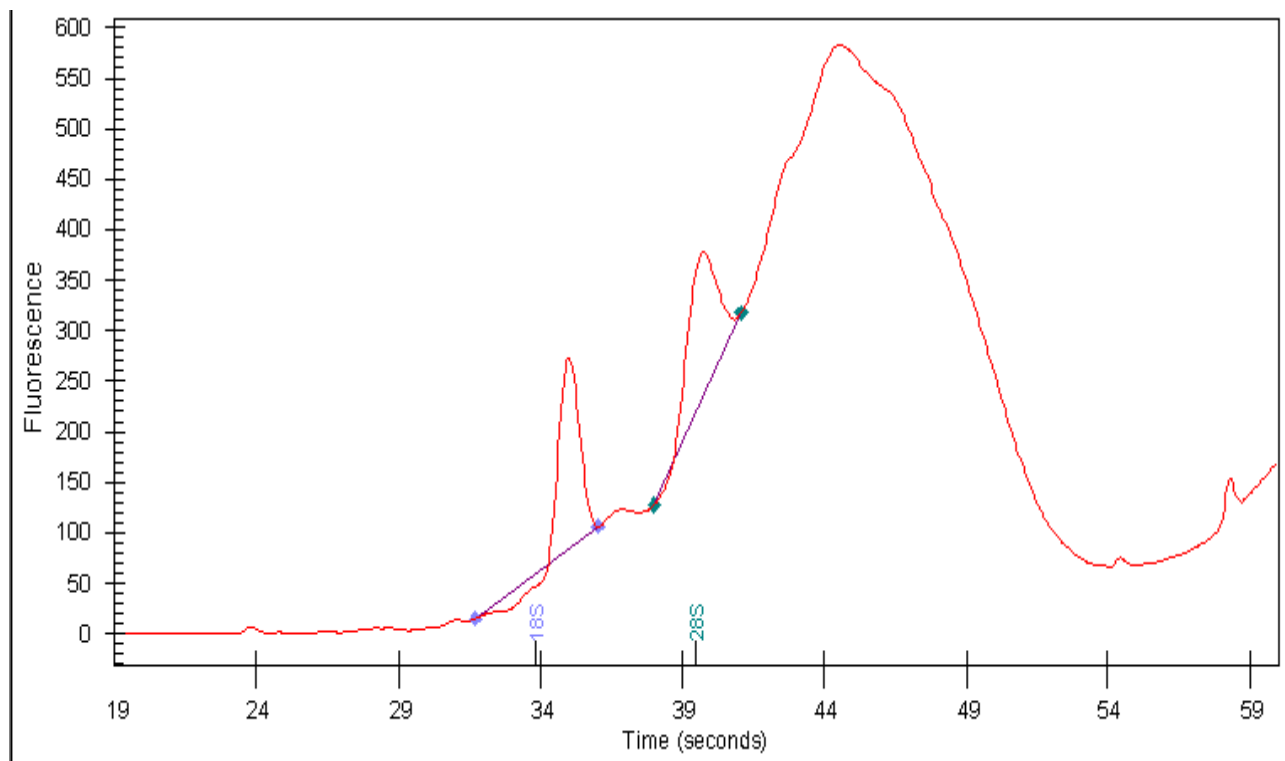
# Noisy Electropherogram

Most Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see RNA Reagent Kit Guide).
Clogged gasket and plastic adapter of priming station	Perform seal test like described in <b>Maintaining the Chip Priming Station—271</b> . Clean/replace gasket and plastic adapter if necessary.
Chip contaminated.	Wear powder-free gloves only.  Don't touch the underside of the chip.  Don't touch the wells of the chip.  Clean the electrodes.  Load the chip immediately after taking it out of its sealed bag.
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run.  Remove vibration devices, such as vortexers and vacuum pumps, from bench.

Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Dye concentration too low.	Use dye concentration according to the RNA Reagent Kit Guide. Let the dye warm up to room temperature before preparing the gel-dye mix.
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> . If the power supply is defective, call Agilent Technologies.

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## Broad Peaks



Show me how to solve **Broad Peaks**

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# Broad Peaks

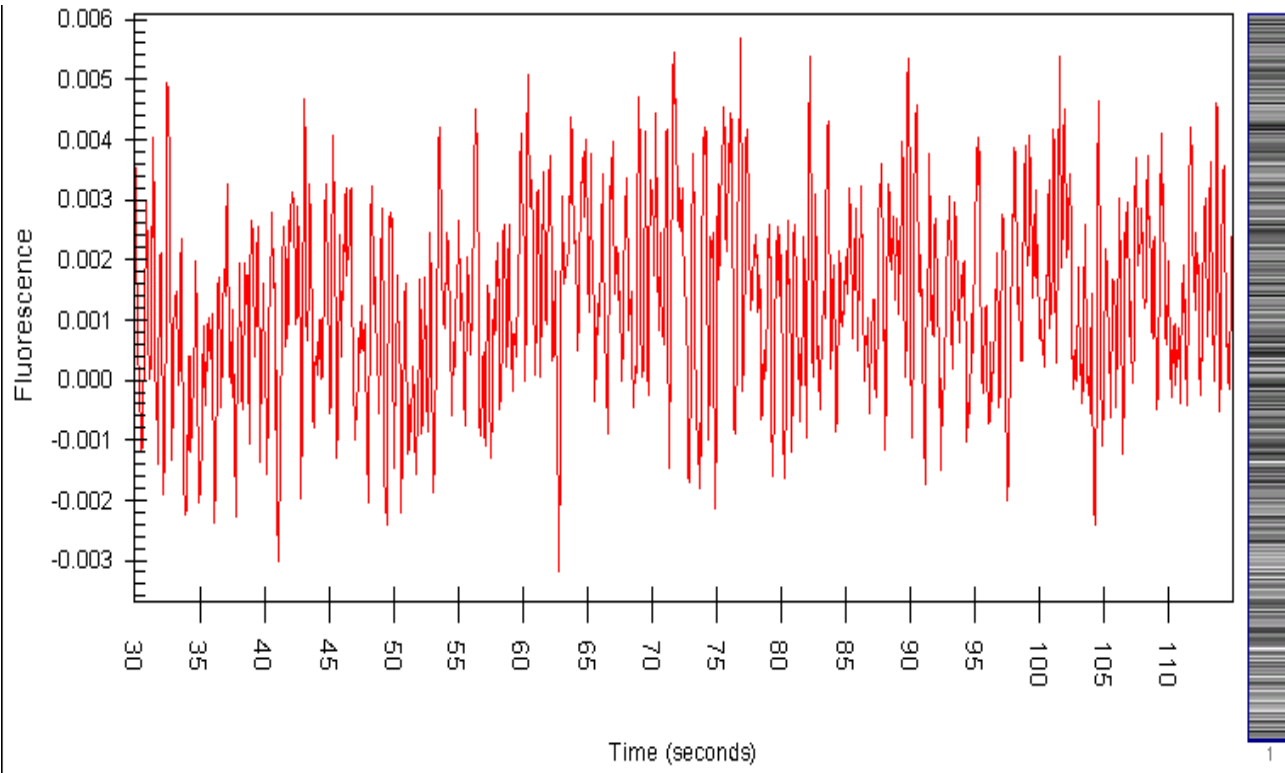
Most Probable Causes	Solution
Leak current due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b> .  Replace electrode cartridge.
Electrodes contaminated with RNAses.	Clean electrodes with RNaseZAP.  Follow decontamination procedure, see <b>Maintenance—246</b> .
Clogged gasket and plastic adapter of priming station	Perform seal test like described in <b>Maintaining the Chip Priming Station—271</b> . Clean/replace gasket and plastic adapter if necessary.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see RNA Reagent Kit Guide).
Dye concentration too high.	Use dye concentration according to the Reagent Kit Guide.  Let the dye warm up to room temperature before preparing the gel-dye mix.



Probable Causes	Solution
Clogged channel due to too high sample concentration in previous lane.	Use sample concentration according to the RNA Reagent Kit Guide.
Particles of protective foam pad on electrode cartridge.	Make sure to remove foam particles of the electrode cartridge before use.
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> . If the power supply is defective, call Agilent Technologies.

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# Missing Peaks



Show me how to solve **Missing Peaks**

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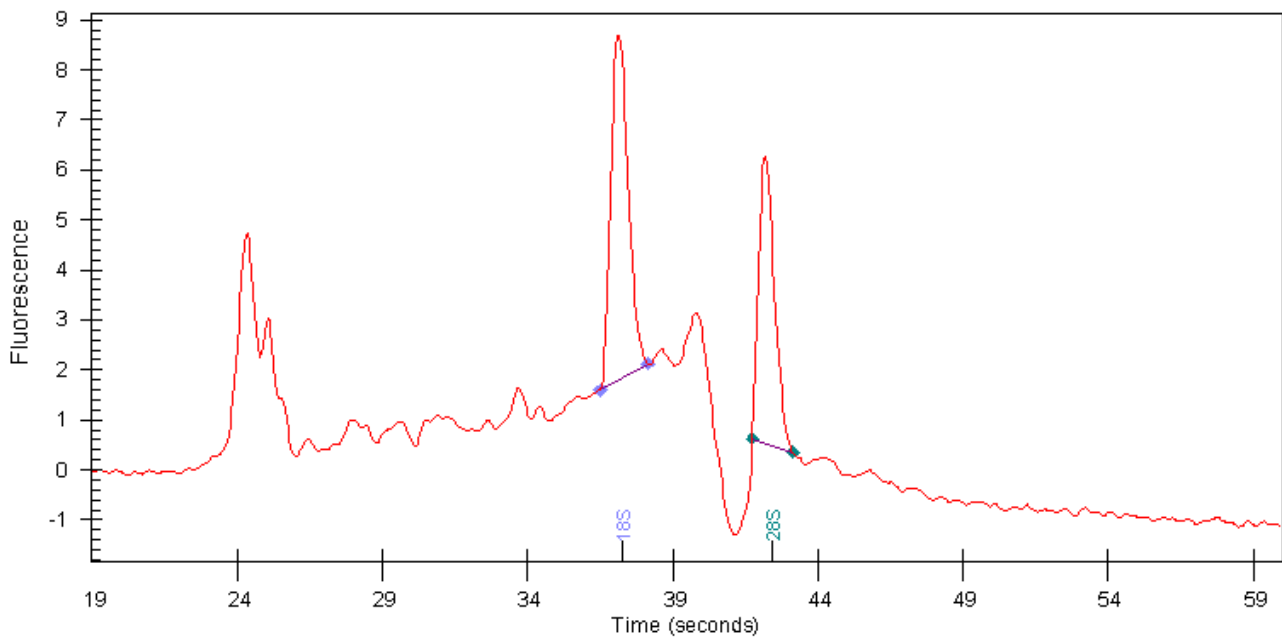
# Missing Peaks

Most Probable Causes	Solution
Laser defective.	Check laser using the <b>Hardware Diagnostics—48</b> .  If the laser is defective, call Agilent Technologies.
No ladder/samples in wells.	Use a new chip. Pipette ladder/sample in all wells
Wrong settings of chip priming station.	For the correct position of the syringe clip and base plate please refer to the Reagent Kit Guide.
Leak current due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b> .  Replace electrode cartridge.

Probable Causes	Solution
Chip pipetting error.	<p>Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Holding the pipette at a slight angle will ensure proper dispensing of the liquid.</p> <p>Use appropriate pipette and tips.</p>
Dye concentration too low.	<p>Use dye concentration according to the Reagent Kit Guide.</p> <p>Let the dye warm up to room temperature before preparing the gel-dye mix.</p>
Least Probable Causes	Solution
Autofocus failure.	<p>Check autofocus by means of the <b>Hardware Diagnostics—48</b>.</p> <p>If autofocus fails, call Agilent Technologies.</p>
High voltage power supply defective.	<p>Check high voltage power supply using the <b>Hardware Diagnostics—48</b>.</p> <p>If the power supply is defective, call Agilent Technologies.</p>

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# Poor Baseline: Dips



Show me how to solve **Poor Baseline: Dips**

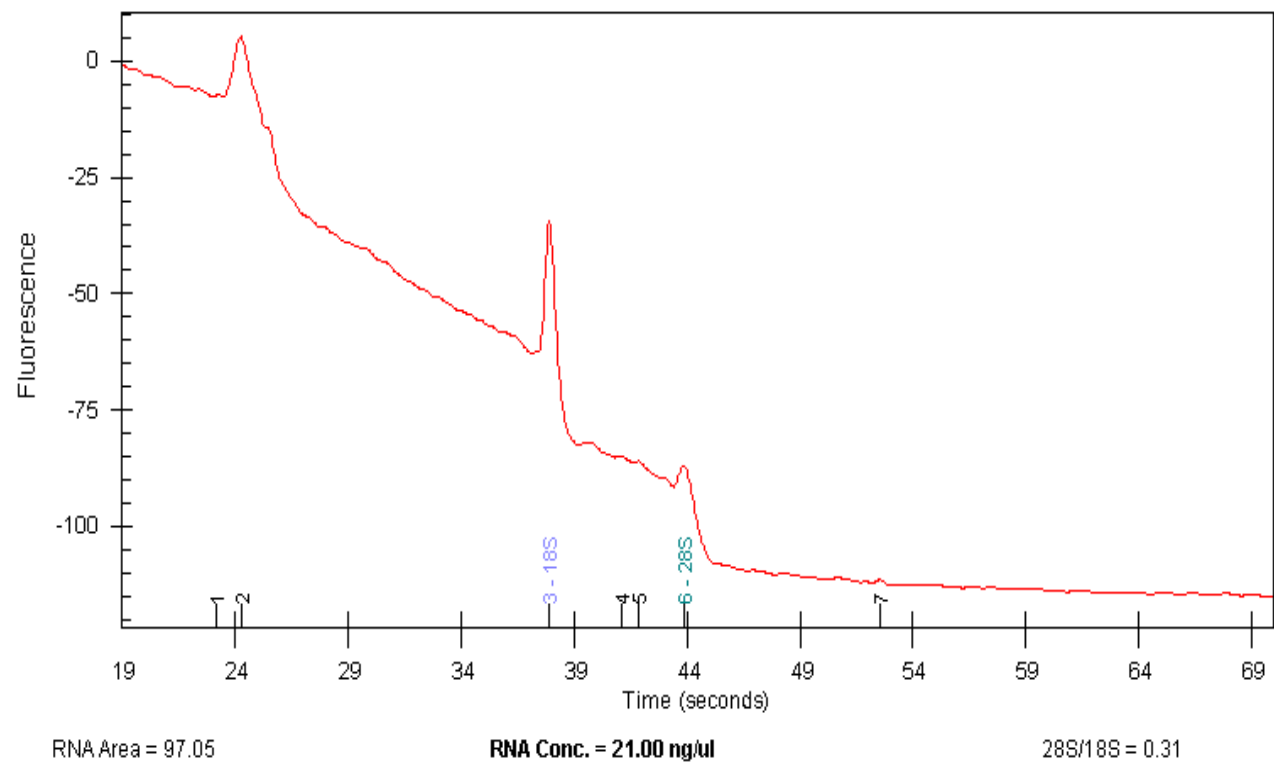
Back to **Symptoms**

# Poor Baseline: Dips

Probable Causes	Solution
Too high sample concentration.	Use sample concentration according to the RNA Reagent Kit Guide.
Sample impurities: e.g. genomic DNA, ss DNA, etc.	Check RNA-isolation protocol. If possible, clean up samples.
Probable Causes	Solution
Clogged gasket and plastic adapter of priming station	Perform seal test like described in <b>Maintaining the Chip Priming Station—271</b> . Clean/replace gasket and plastic adapter if necessary.
Least Probable Causes	Solution
Autofocus failure.	Check autofocus by means of the <b>Hardware Diagnostics—48</b> .  If autofocus fails, call Agilent Technologies.

Back to **Symptoms**

# Poor Baseline: Drift



Show me how to solve **Poor Baseline: Drift**

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# Poor Baseline: Drift

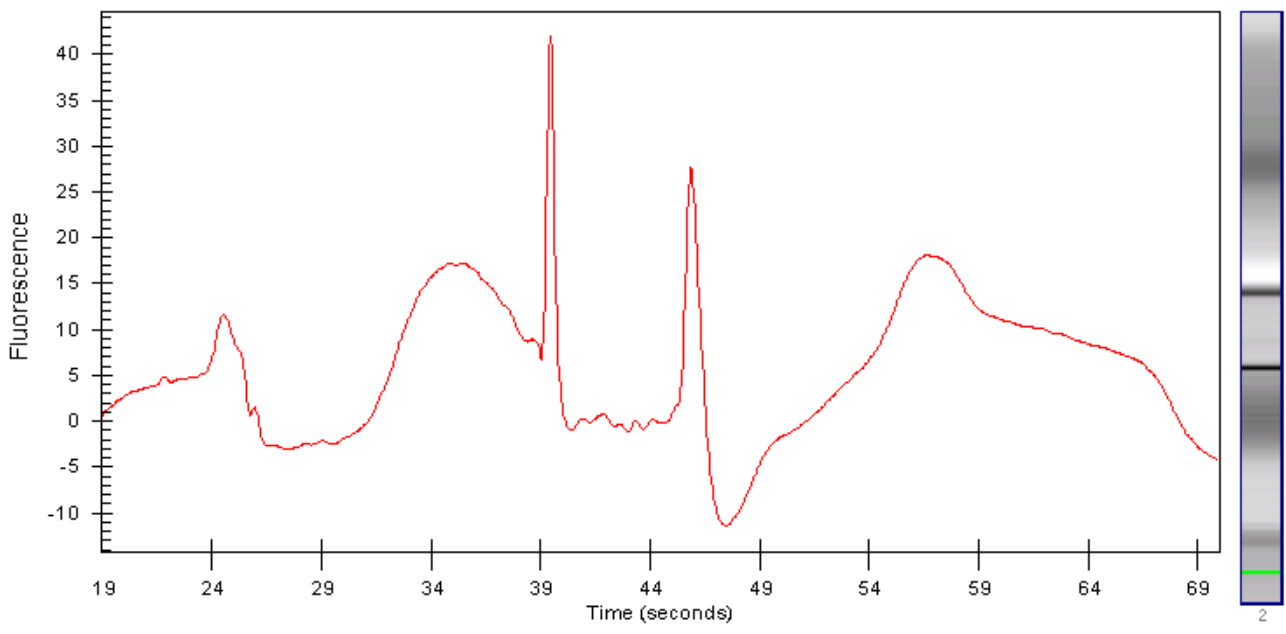
Most Probable Causes	Solution
Leak current due to dirty electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b> .  Replace electrode cartridge.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Dye concentration too low.	Use dye concentration according to the Reagent Kit Guide.  Let the dye warm up to room temperature before preparing the gel-dye mix.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Clogged gasket and plastic adapter of priming station	Perform seal test like described in <b>Maintaining the Chip Priming Station—271</b> . Clean/replace gasket and plastic adapter if necessary.



Probable Causes	Solution
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
Least Probable Causes	Solution
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 Bioanalyzer in thermally stable environment.
High voltage power supply defective.	<p>Check high voltage power supply using the <b>Hardware Diagnostics—48</b>.</p> <p>If the power supply is defective, call Agilent Technologies.</p>

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# Wavy Baseline



Show me how to solve **Wavy Baseline**

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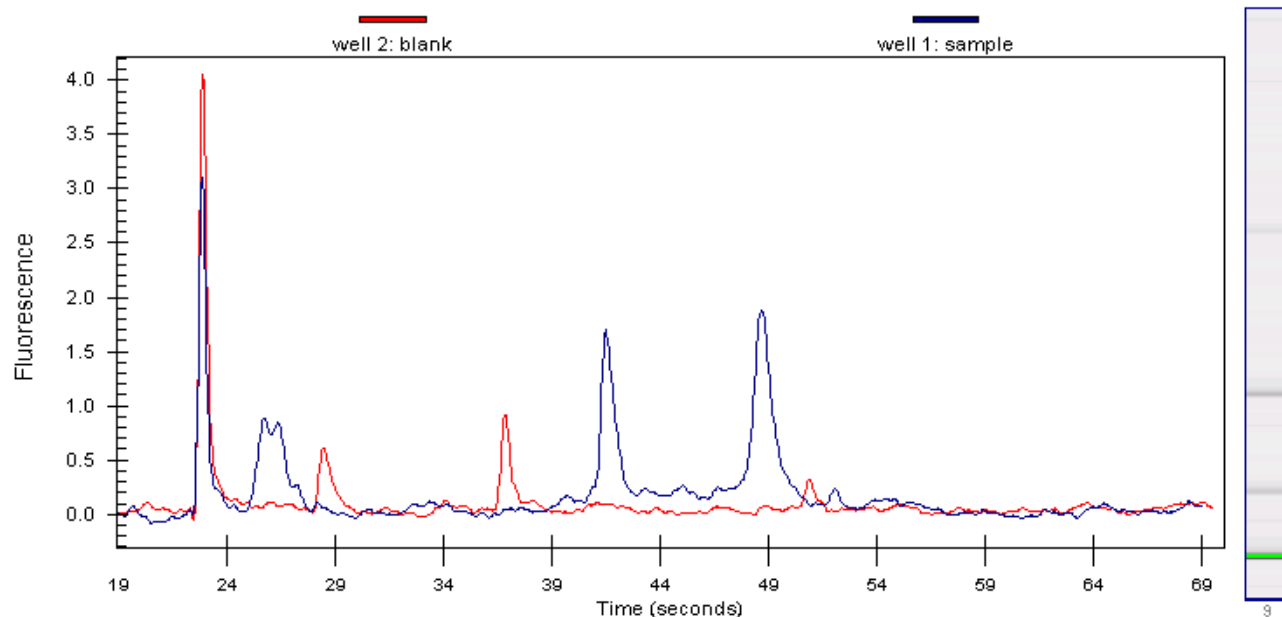
# Wavy Baseline

Most Probable Causes	Solution
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b> .  Replace electrode cartridge.
Clogged gasket and plastic adapter of priming station	Perform seal test like described in <b>Maintaining the Chip Priming Station—271</b> . Clean/replace gasket and plastic adapter if necessary.
Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b> ).  Check if clip and base plate of priming station are in the right position (see RNA Reagent Kit Guide).
Dye concentration too low.	Use dye concentration according to the Reagent Kit Guide.  Let the dye warm up to room temperature before preparing the gel-dye mix.

Least Probable Causes	Solution
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
High voltage power supply defective.	<p>Check high voltage power supply using the <b>Hardware Diagnostics—48</b>.</p> <p>If the power supply is defective, call Agilent Technologies.</p>
Autofocus failure.	<p>Check autofocus using the <b>Hardware Diagnostics—48</b>.</p> <p>If autofocus fails, call Agilent Technologies.</p>

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# Cross Contamination



Show me how to solve **Cross Contamination**

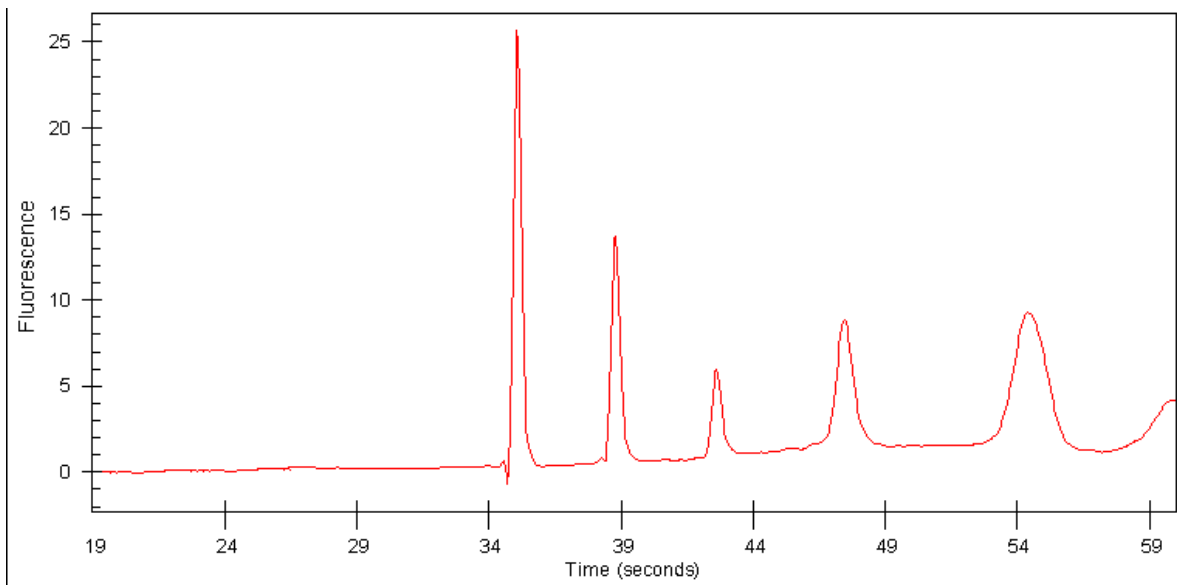
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# Cross Contamination

Most Probable Causes	Solution
Sample concentration too high.	Use sample concentration according to the RNA Reagent Kit Guide.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip and pipette again. Use appropriate pipette and tips.
Probable Causes	Solution
Leak current due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b> . Replace electrode cartridge.

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# Late Migration of RNA Ladder or Samples



Show me how to solve **Late Migration of RNA Ladder or Sample**

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# Late Migration of RNA Ladder or Sample

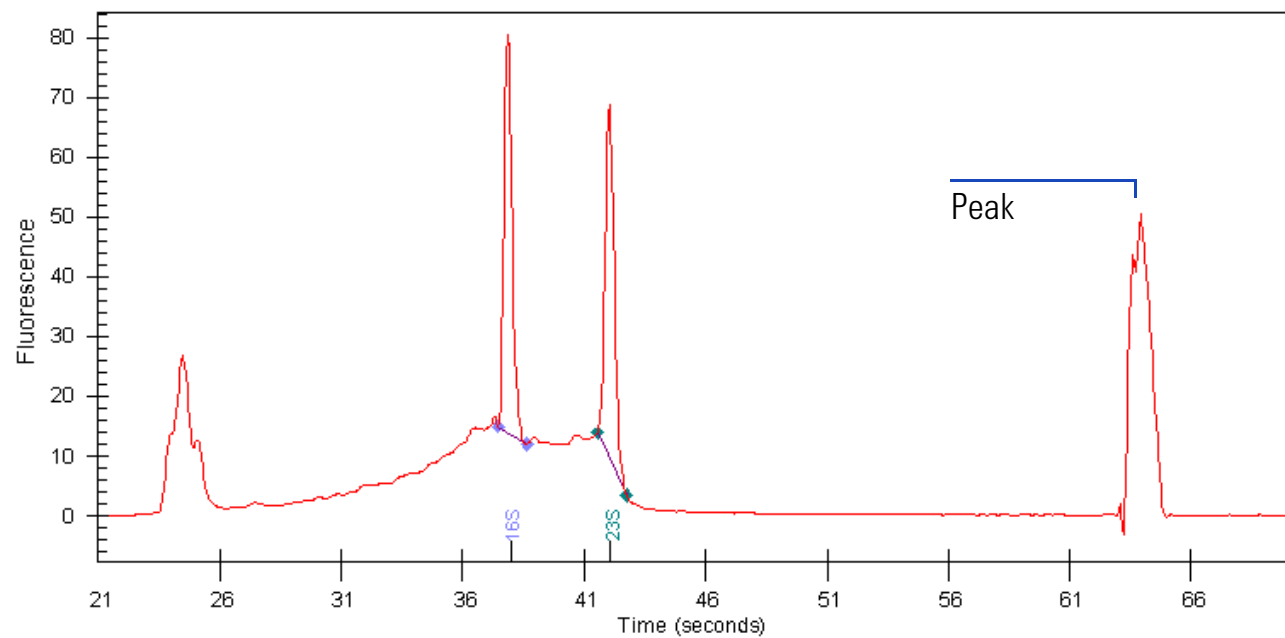
Most Probable Causes	Solution
Clogged gasket and plastic adapter of priming station	Perform seal test like described in <b>Maintaining the Chip Priming Station—271</b> . Clean/replace gasket and plastic adapter if necessary.
Probable Causes	Solution
Vortex adapter not connected tightly.	Press vortex adapter tightly on mount (vortex adapter must not rock). Only use the IKA vortexer. Replace adapter if necessary.

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# List of RNA Electropherograms

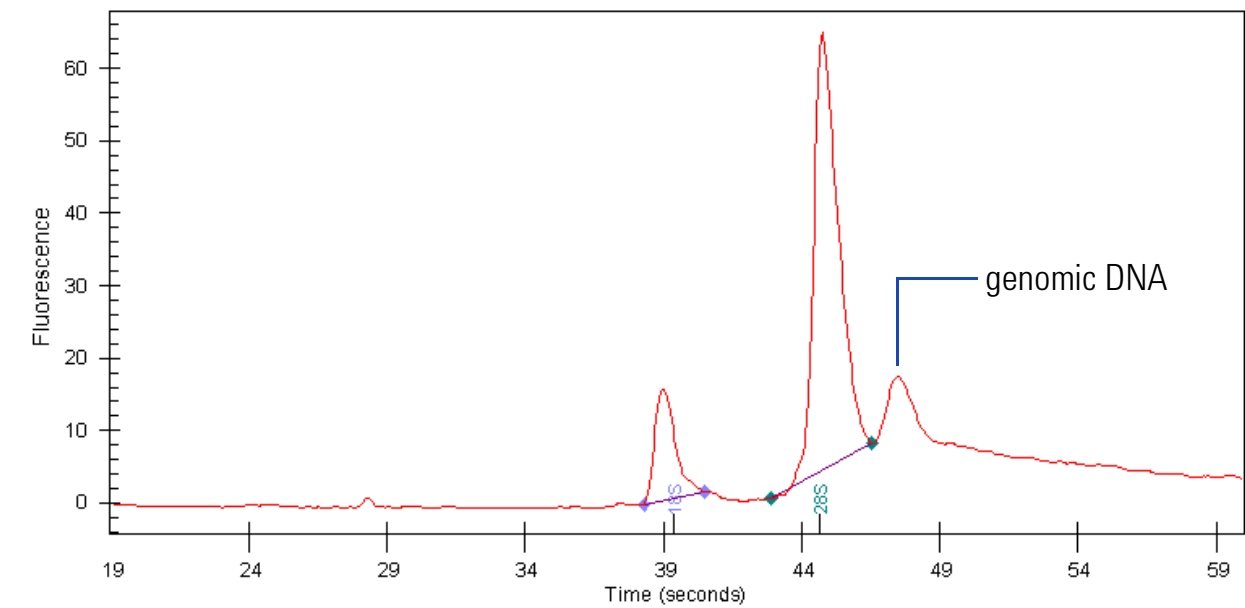
## Additional Sample or Ladder Peaks



Show me how to solve **Additional Sample or Ladder Peaks**

Back to **Symptoms**

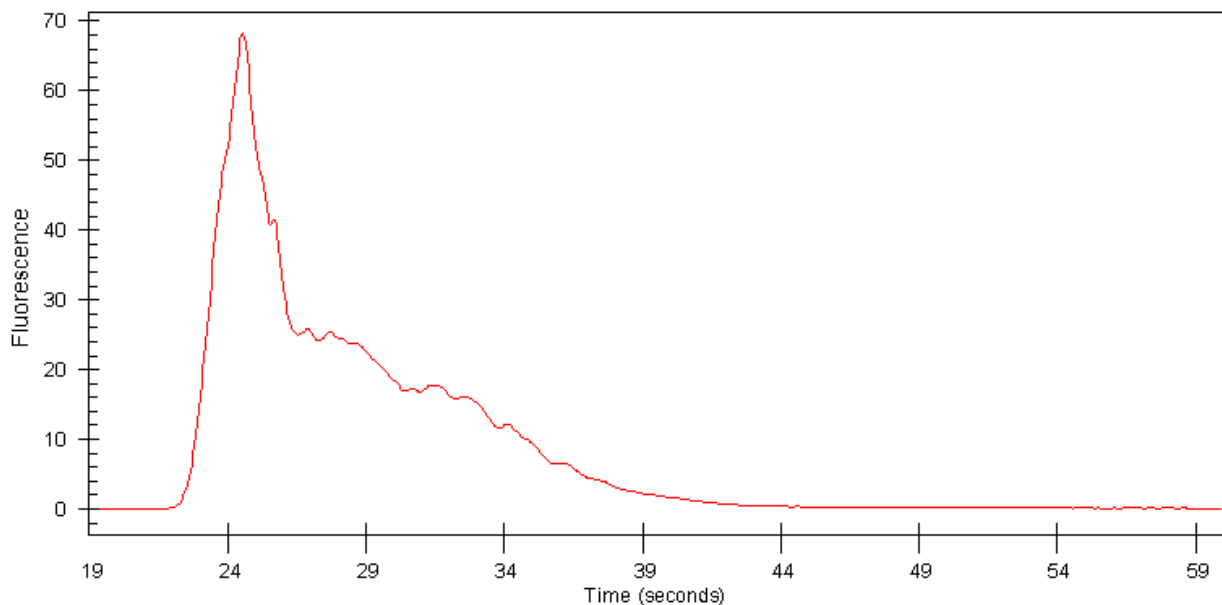
# Genomic DNA Contamination



Show me how to solve **Genomic DNA Contamination**

Back to **Symptoms**

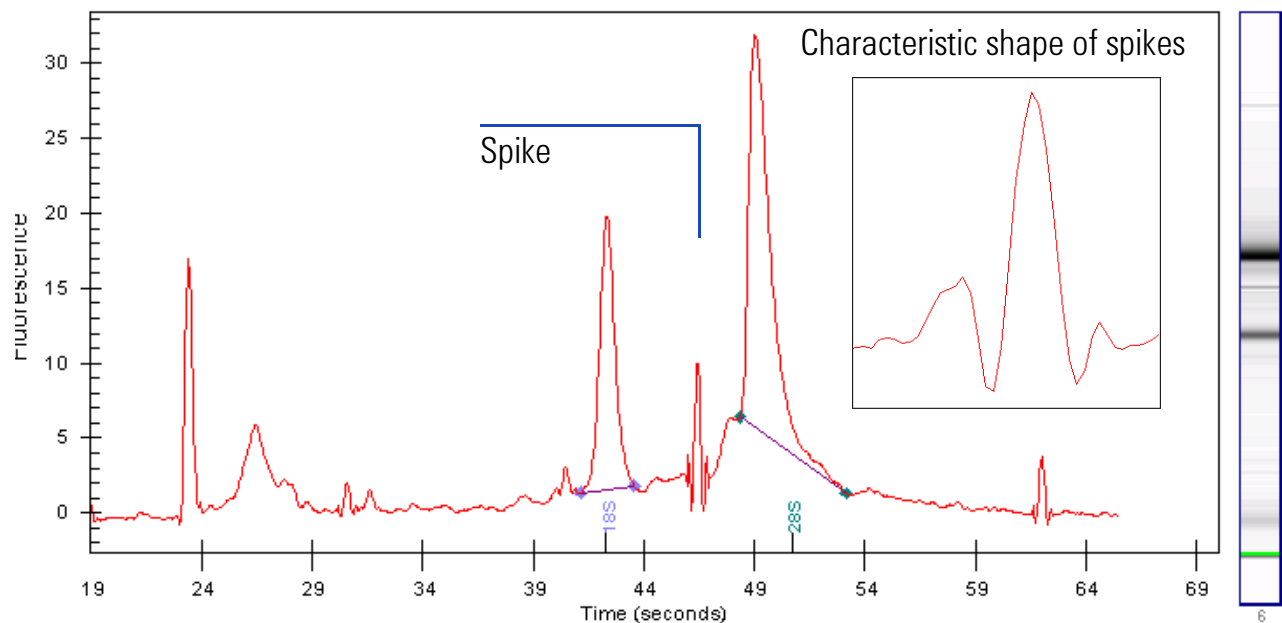
# Degraded RNA Ladder and/or Samples



Show me how to solve **Degraded RNA Ladder and/or Samples**

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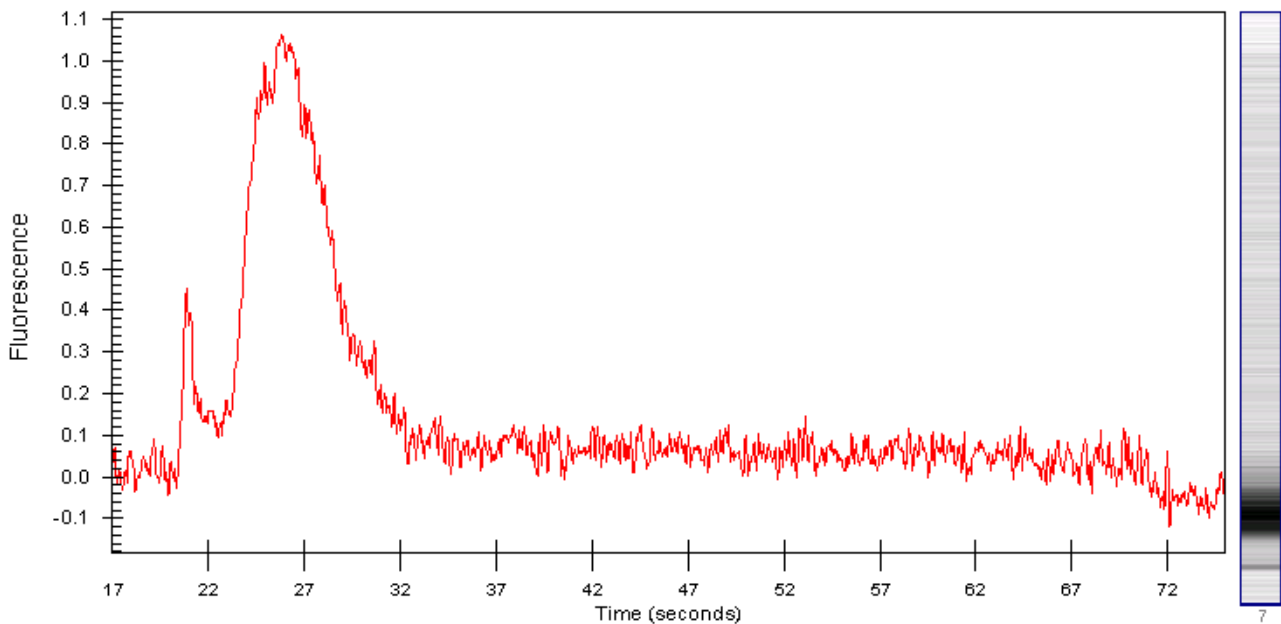
# Spikes/Glitches



Show me how to solve **Spikes/Glitches**

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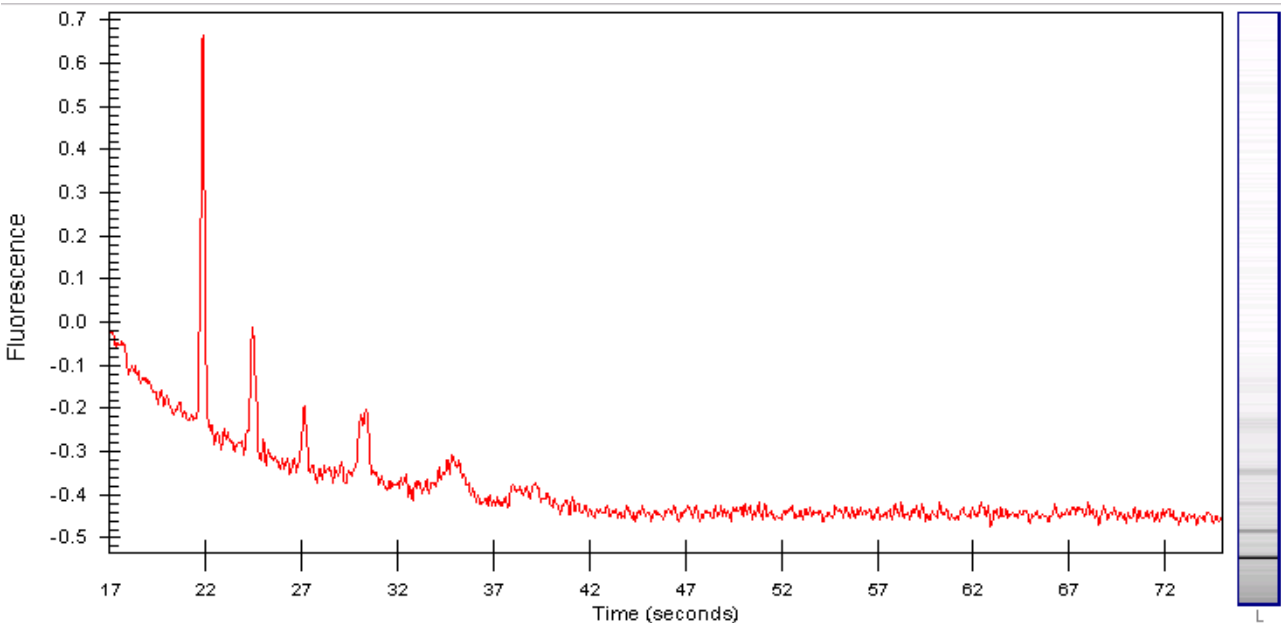
# Poor Sensitivity



Show me how to solve **Poor Sensitivity**

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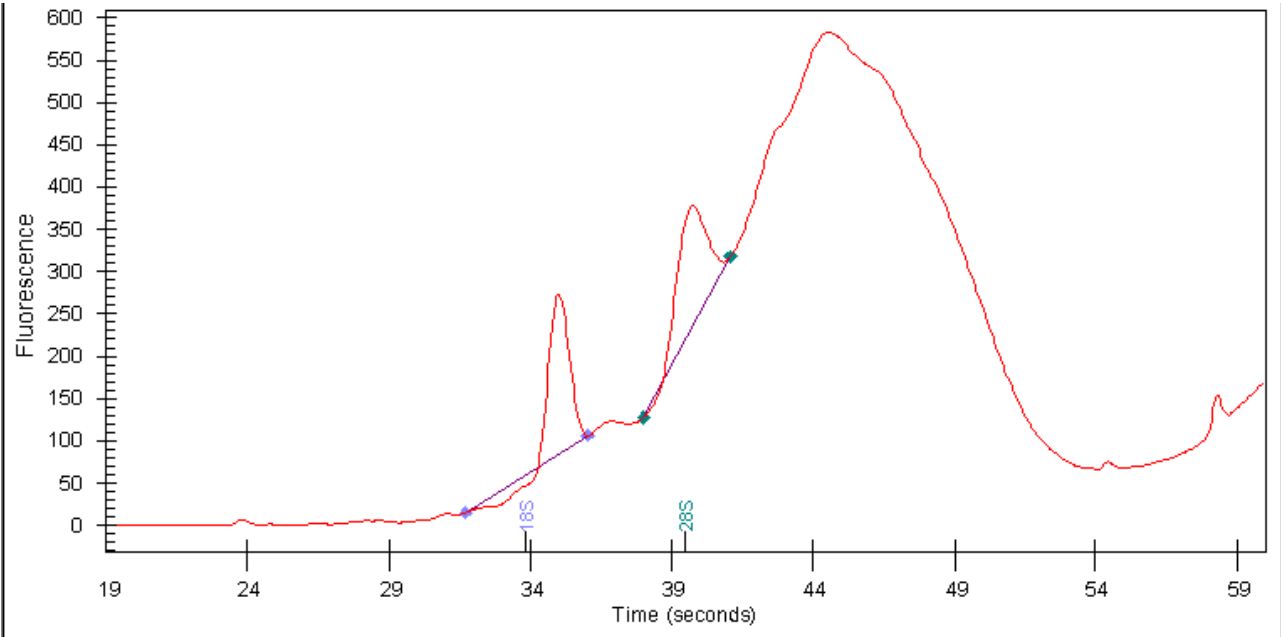
# Noisy Electropherogram



Show me how to solve Noisy Electropherogram

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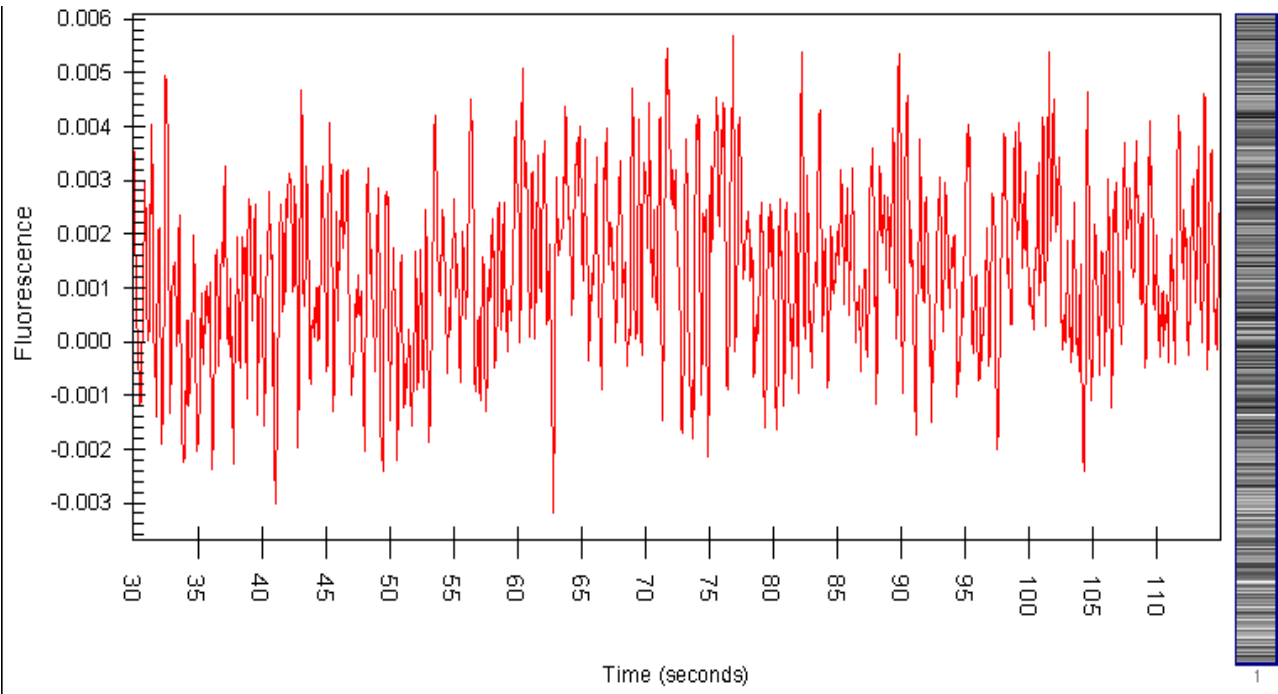
# Broad Peaks



Show me how to solve **Broad Peaks**

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# Missing Peaks

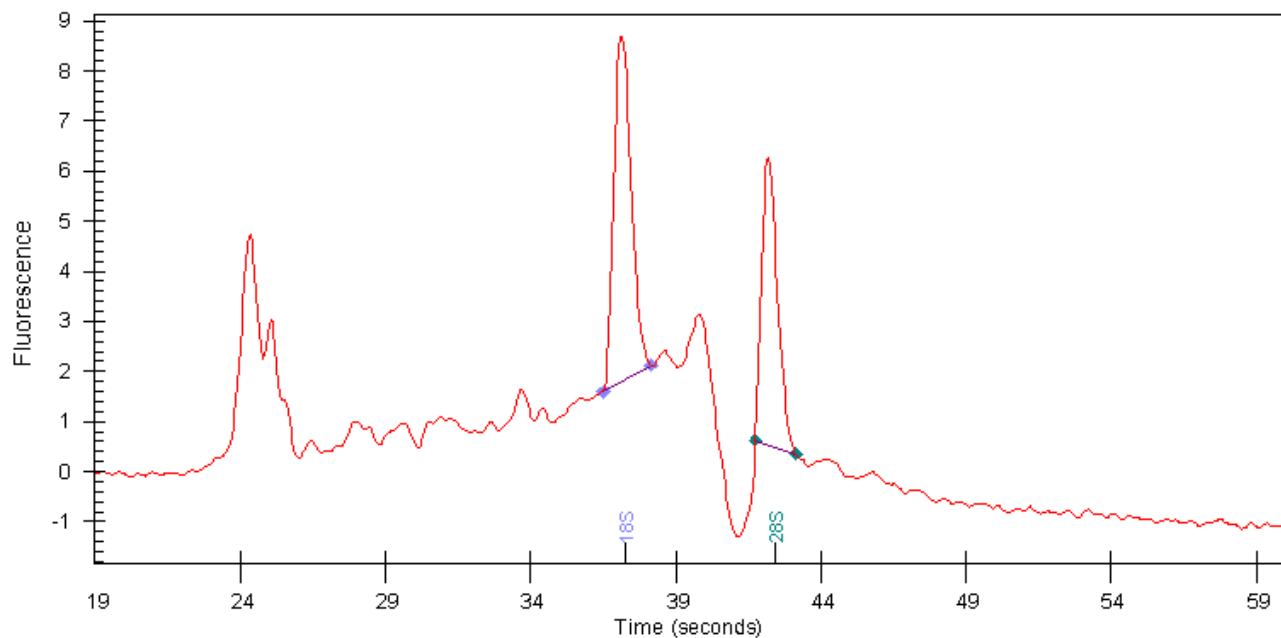


Show me how to solve **Missing Peaks**

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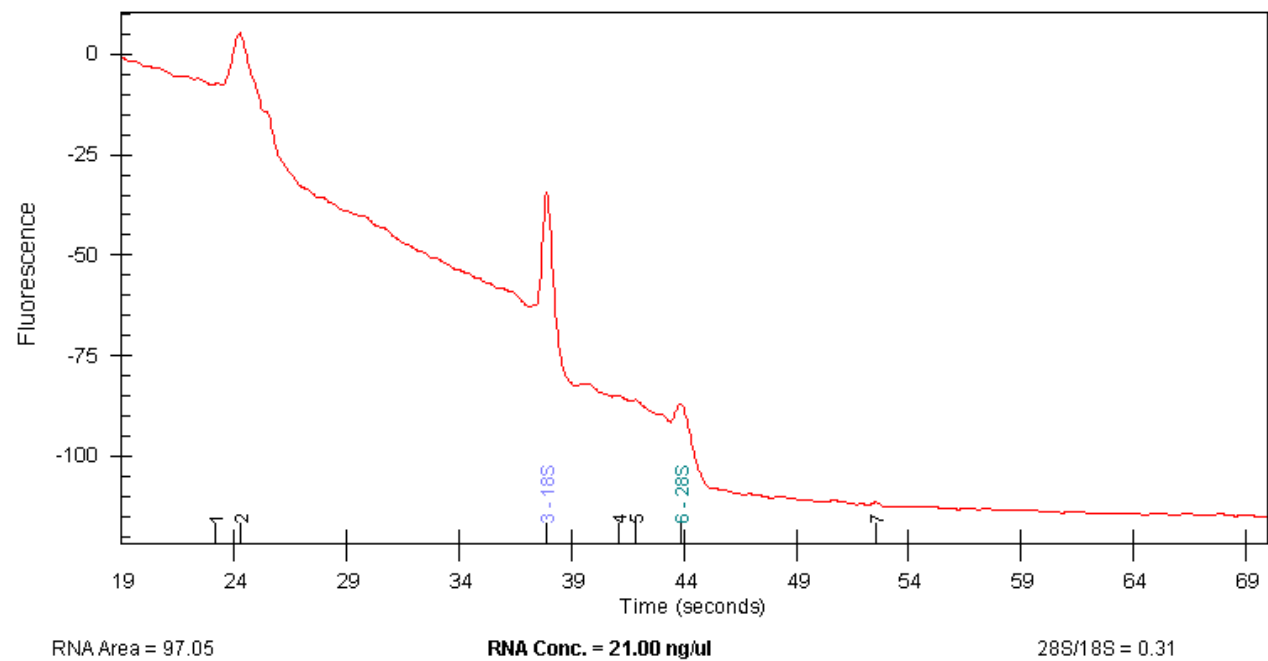
## Poor Baseline: Dips



Show me how to solve **Poor Baseline: Dips**

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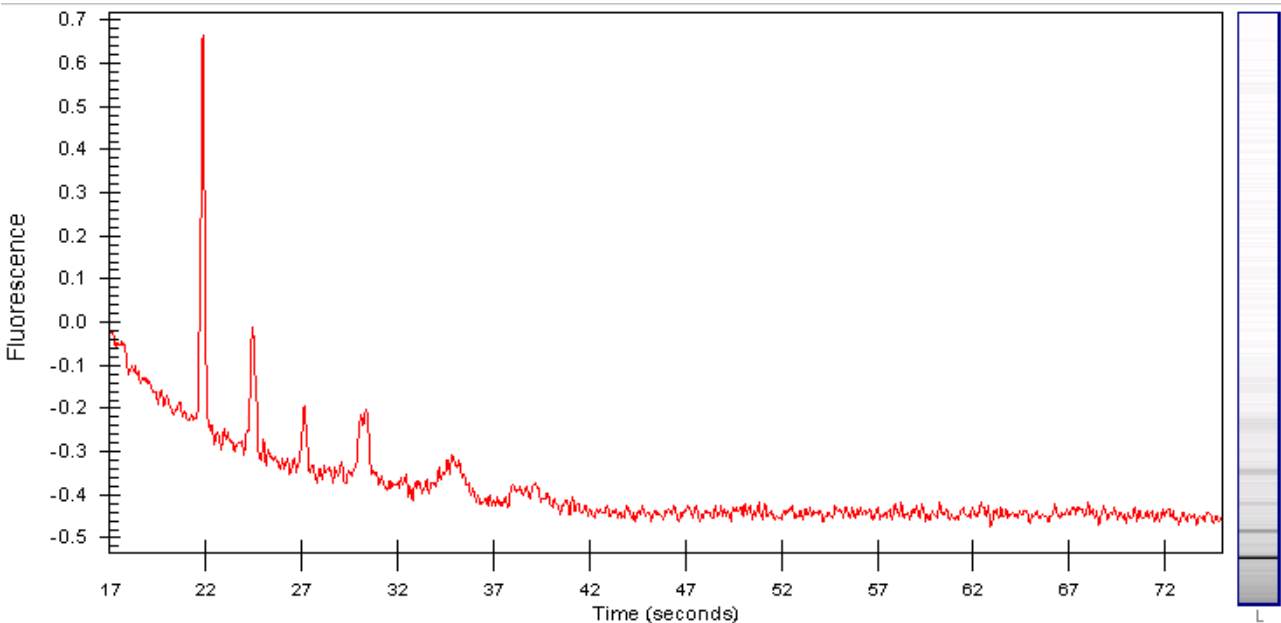
Poor Baseline: Drift



Show me how to solve **Poor Baseline: Drift**

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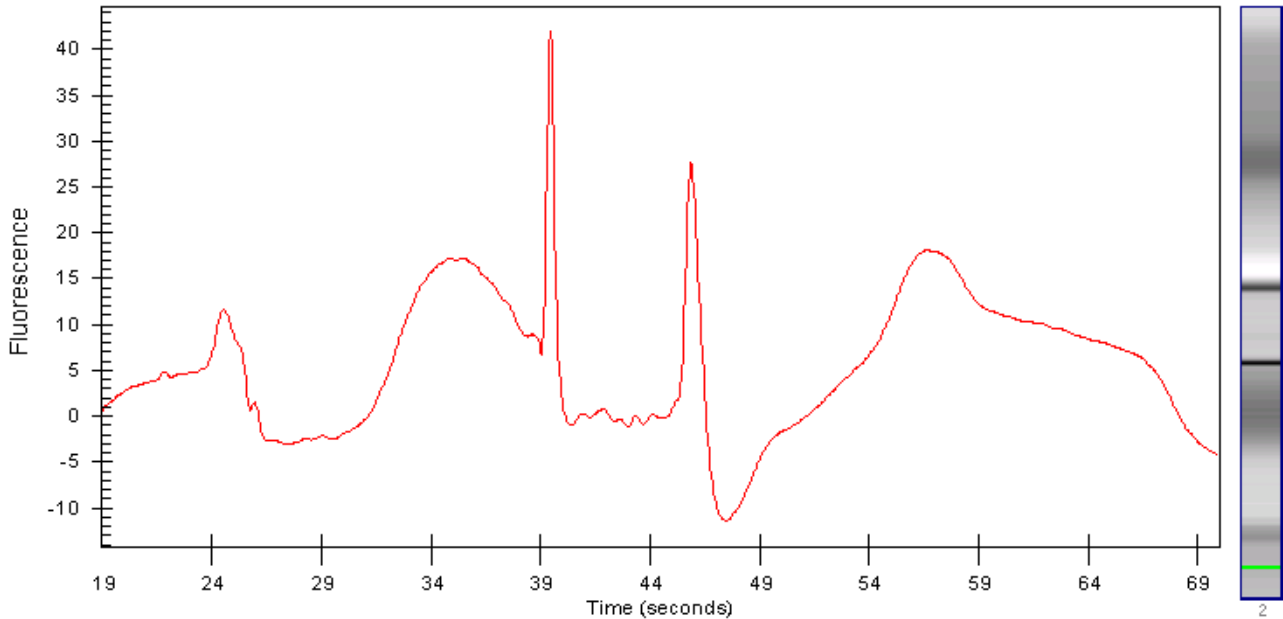
# Noisy Electropherogram



Show me how to solve **Noisy Electropherogram**

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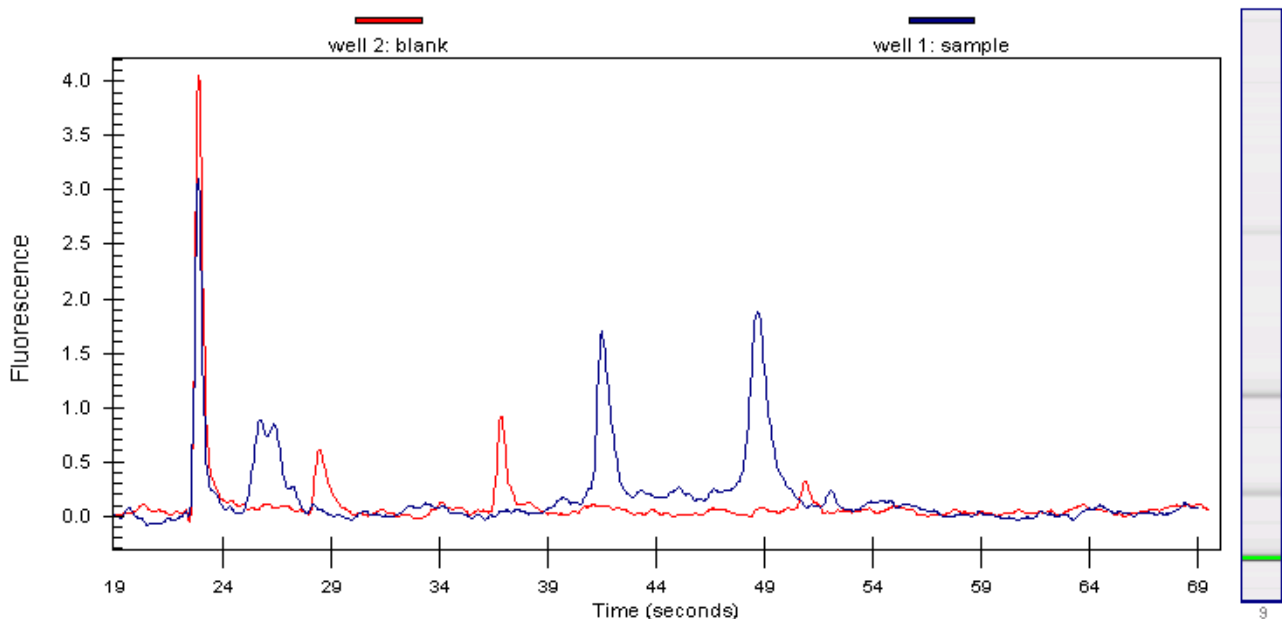
# Wavy Baseline



Show me how to solve **Wavy Baseline**

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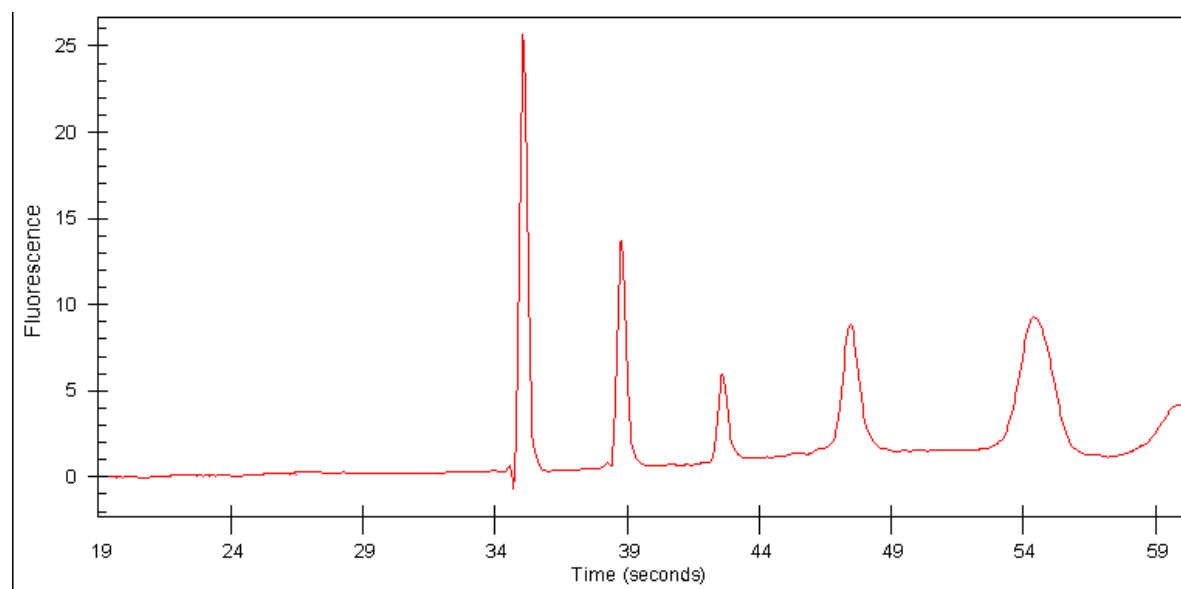
# Cross Contamination



Show me how to solve **Cross Contamination**

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# Late Migration of RNA Ladder or Sample



Show me how to solve **Late Migration of RNA Ladder or Sample**

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# Troubleshooting the Protein Application

## Essential Measurement Practices

For hints on how to handle chips and chemicals, see **Essential Measurement Practices—7**.

## Troubleshooting the Protein Application

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Click the  or  button next to the error message to view a help screen that is specific for that error.

Additional information regarding the nature of a problem can often be found in the run log for the data file. Choose Tools > View Log File > Run Log. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by your Agilent 2100 Bioanalyzer might not be what you expected. To help you find the reason for the discrepancy, see **Symptoms—185**.

For most observations you will find at least one corresponding example, depicting a typical electropherogram or result table. Once you have identified the observation that resembles the outcome of your experiment, you will get a set of assigned causes listed by priority.

The causes are grouped into three levels:

- **most probable cause**
- **probable cause**
- **least probable cause**

A list of solutions that help you to fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by priority.

If you are not able to assign a symptom to your problem, compare your electropherogram with the **List of Protein Electropherograms—231**.



# Symptoms

Click the icon to see an example, or go straight to the troubleshooting hints.

Clogged spin filters—187

Too High Quantitation Results—188

Too Low Quantitation Results—190

Wrong Sizing Result—191

Poor Chip Performance—193



Apparently Short Run Time—195



Additional Sample or Ladder Peaks—197



Low or Missing Upper Marker in Ladder—200



Low or Missing Upper Marker in Sample—202



High Lower Marker Variability—205



No Peaks—207



Spikes—209



Poor Reproducibility—212



No or Low Sample Peaks—215



Apparently Missing Sample Peak—218



Low Ladder Peaks—220



Wrong Alignment of Ladder Peaks—222



Broad Peaks—224



Dips—229



Cross Contamination—227

List of Protein Electropherograms—231

# Clogged spin filters

Most Probable Causes	Solution
Gel was centrifuged at too low g-value.	Refer to the Protein Reagent Kit Guide for proper centrifuge settings.
Cooled centrifuge was used for preparation of gel-dye mix and/or destaining solution.	Repeat centrifugation step without cooling.
Least Probable Causes	Solution
Particles in the gel-dye mix and/or destaining solution.	Repeat the preparation of the gel-dye mix and/or destaining solution.
	Wear powder-free gloves only.

Back to **Symptoms**

# Too High Quantitation Results

Most Probable Causes	Solution
Upper marker wrongly assigned.	Check assignment of upper marker.
Diluted samples are too old.	Use diluted samples within one day.
Sample buffer and/or Denaturing Solution not handled according to the instructions.	For proper preparation and storage of the sample buffer and denaturing solution, refer to the Protein Ragent Kit Guide.
Pipetting error during preparation of mixtures.	Check dilution procedure.  Check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid.  Use appropriate pipette and tips.
Probable Causes	Solution
Samples not completely denaturated.	Use fresh sample aliquot. Heat sample/ denaturing solution for 5 min at 100°C.
Sample/denaturing solution are dried out.	Sample/denaturing solution were denaturated in 1.5 mL tubes. Use 0.5 mL tubes for denaturing.

Least Probable Causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 10 minutes.

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# Too Low Quantitation Results

Most Probable Causes	Solution
Upper marker wrongly assigned.	Check assignment of upper marker.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable Causes	Solution
Sample concentration too high.	Use sample concentration according to the Protein Reagent Kit Guide. Don't forget to dilute samples with deionized water after heat denaturation.
Diluted sample are too old.	Use diluted samples within one day.
Least Probable Causes	Solution
Loaded chip kept too long before run.	Prepared chips must be used within 10 min.

Back to **Symptoms**

# Wrong Sizing Result

Most Probable Causes	Solution
Ladder degraded.	Refer to the Reagent Kit guide for proper ladder storage. Optional: prepare ladder aliquot.  Use a fresh ladder aliquot.
Upper and/or lower marker wrongly assigned.	Store Sample Buffer/Denaturing Solution according to the instructions given in the Reagent Kit Guide.  See <b>Low or Missing Upper Marker in Ladder—200</b>
Ladder peaks wrongly assigned.	Check assignment of ladder peaks.  See <b>Wrong Alignment of Ladder Peaks—222</b> for details.
Protein ladder not properly denaturated.	Use fresh ladder aliquot. Heat ladder for 5 min at 100°C.
Probable Causes	Solution
Protein ladder not properly denaturated.	Use fresh ladder aliquot. Heat ladder for 5 min at 100°C.

Least Probable Causes	Solution
Vibration of Agilent 2100 Bioanalyzer.	<p>Don't touch Agilent 2100 Bioanalyzer during a run.</p> <p>Remove vibration devices, such as vortexers and vacuum pumps, from bench.</p>
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 Bioanalyzer in thermally stable environment.
High voltage power supply defective.	<p>Check high voltage power supply using the <b>Hardware Diagnostics—48</b>.</p> <p>If the power supply is defective, call Agilent Technologies.</p>

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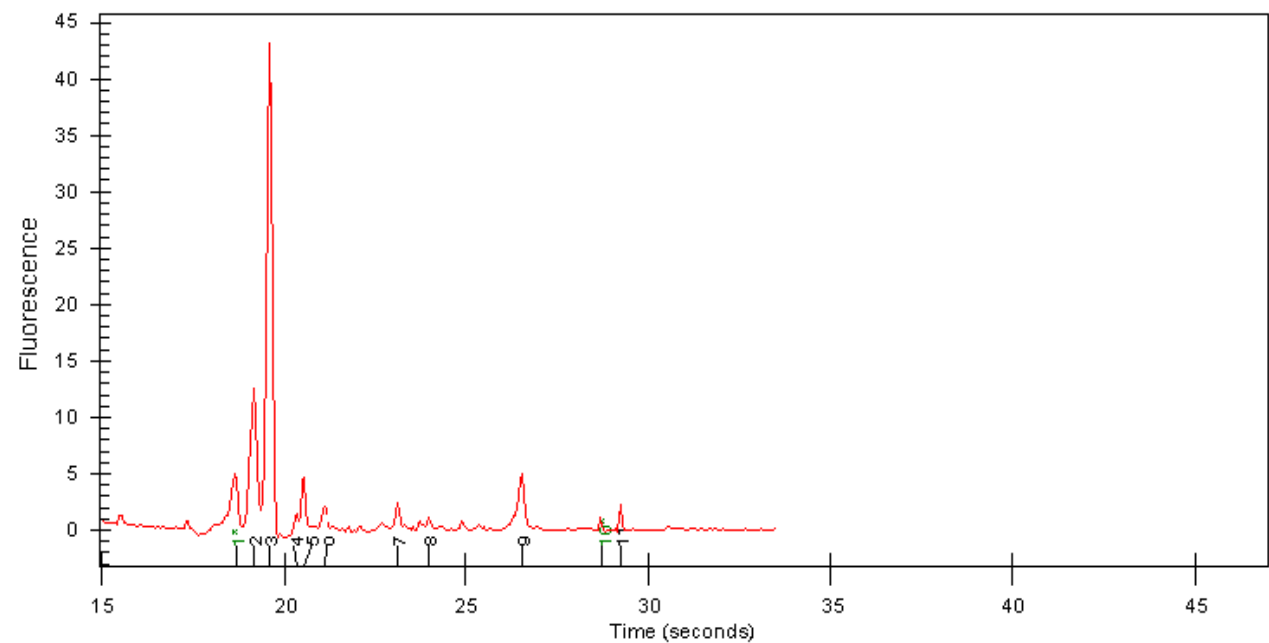
# Poor Chip Performance

Most Probable Causes	Solution
Chip not properly primed. Air bubble in chip.	<p>Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b>).</p> <p>Check if clip and base plate of priming station are in the right position (see Protein Reagent Kit Guide).</p>
Amount of liquid pipetted is too low or chip is empty.	<p>Check Reagent Kit Guide on amount of liquid to be pipetted. Fill unused wells with ladder or sample replicate.</p> <p>Check calibration of pipette.</p>
Chip pipetting error.	<p>Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid.</p> <p>Use appropriate pipette and tips.</p>

Probable Causes	Solution
Chip preparation was done with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature before use.
Chips were stored in the fridge/freezer.	Prepare a new chip. Store chips at room temperature.
Least Probable Causes	Solution
High voltage power supply defective.	<p>Check high voltage power supply using the <b>Hardware Diagnostics—48</b>.</p> <p>If the power supply is defective, call Agilent Technologies.</p>

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# Apparently Short Run Time



Show me how to solve Apparently Short Run Time

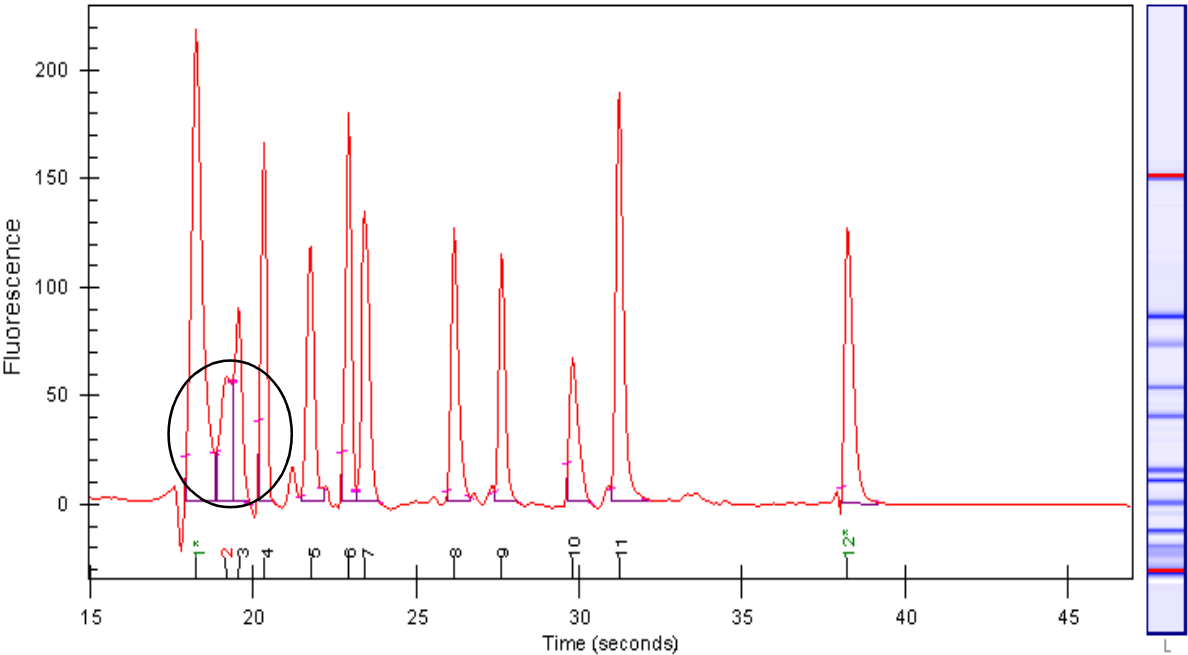
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# Apparently Short Run Time

Most Probable Causes	Solution
Low intensity of upper marker in the ladder. They were not assigned correctly by the software.	<p>To correct for wrong selected upper marker in ladder, set upper marker manually. If necessary, adjust peak find settings. If peaks are detected that are not part of the ladder, exclude them.</p> <p>For better upper marker identification:</p> <p>Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker.</p> <p>See</p> <p><b>Low or Missing Upper Marker in Ladder—201</b></p> <p>for probable causes.</p>

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# Additional Sample or Ladder Peaks



Show me how to solve **Additional Sample or Ladder Peaks**

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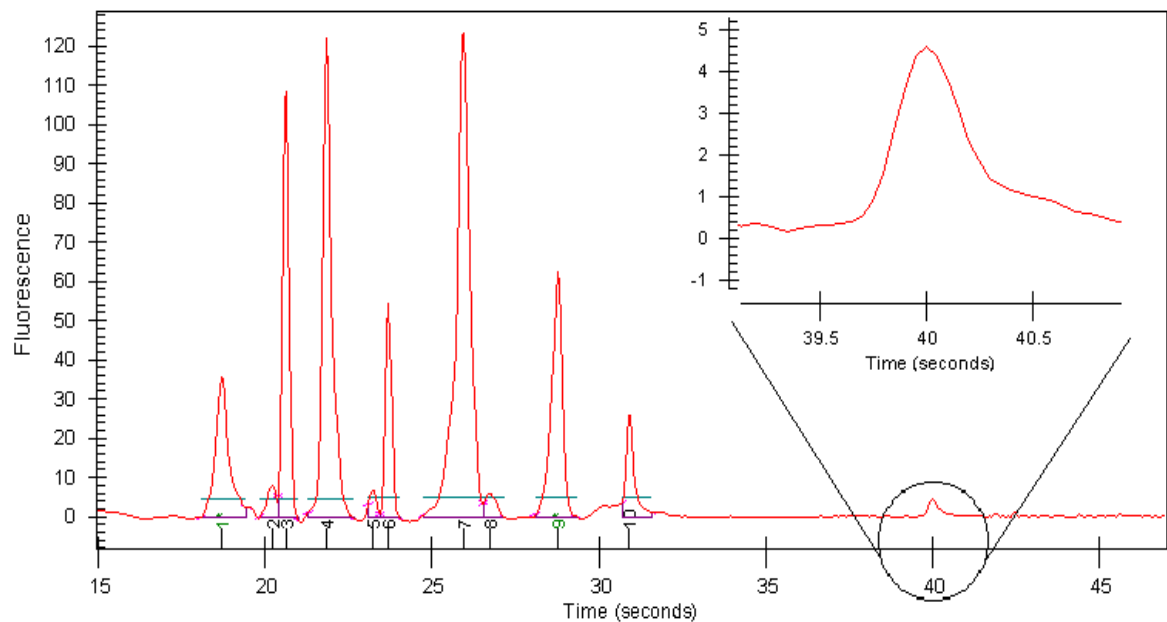
# Additional Sample or Ladder Peaks

Most Probable Causes	Solution
Sample or ladder not denaturated properly.	Use fresh sample aliquot. Heat sample/ denaturating solution and ladder for 5 min at 100°C
Sample/denaturating solution and/or ladder are dried out during denaturation.	Sample/denaturating solution and/or ladder were denaturated in 1.5 mL tubes. Use 0.5 mL tubes for denaturating
Chip contaminated.	Wear powder-free gloves only.
Dust particles in separation channels.	Don't touch the wells of the chip. Clean the electrodes. See <b>Maintenance—246</b> for additional information. Load the chip immediately after taking it out of its sealed bag.

Probable Causes	Solution
Ladder degraded.	Refer to the Protein Reagent Kit Guide for proper ladder storage.  Optional: Prepare ladder aliquots.  Use a new aliquot.
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run.  Remove vibration devices, such as vortexers and vacuum pumps, from bench.

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# Low or Missing Upper Marker in Ladder



Show me how to solve **Low or Missing Upper Marker in Ladder**

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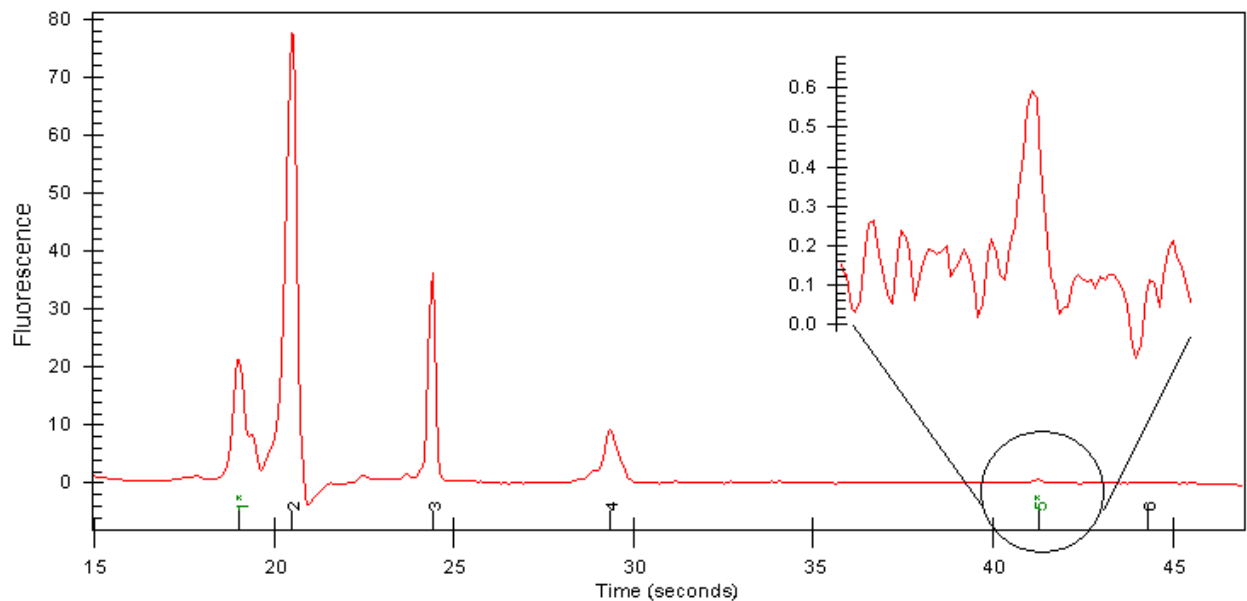


# Low or Missing Upper Marker in Ladder

Most Probable Causes	Solution
Ladder degraded.	<p>For correct ladder storage and denaturation, refer to the Protein Reagent Kit Guide.</p> <p>To correct for wrong selected upper marker, set upper marker manually. If necessary, adjust peak find settings. If peaks are detected that are not part of the ladder, exclude them.</p> <p>For better upper marker identification:</p> <p>Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker.</p>
Diluted ladder is too old.	Use diluted ladder within one day.
Probable Causes	Solution
Ladder not denaturated properly.	Use fresh ladder aliquot. Heat ladder for 5 min at 100°C
Ladder dried out during denaturation.	Ladder was denaturated in 1.5 mL tubes. Use 0.5 mL tubes for denaturing.

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# Low or Missing Upper Marker in Sample



Show me how to solve **Low or Missing Upper Marker in Sample**

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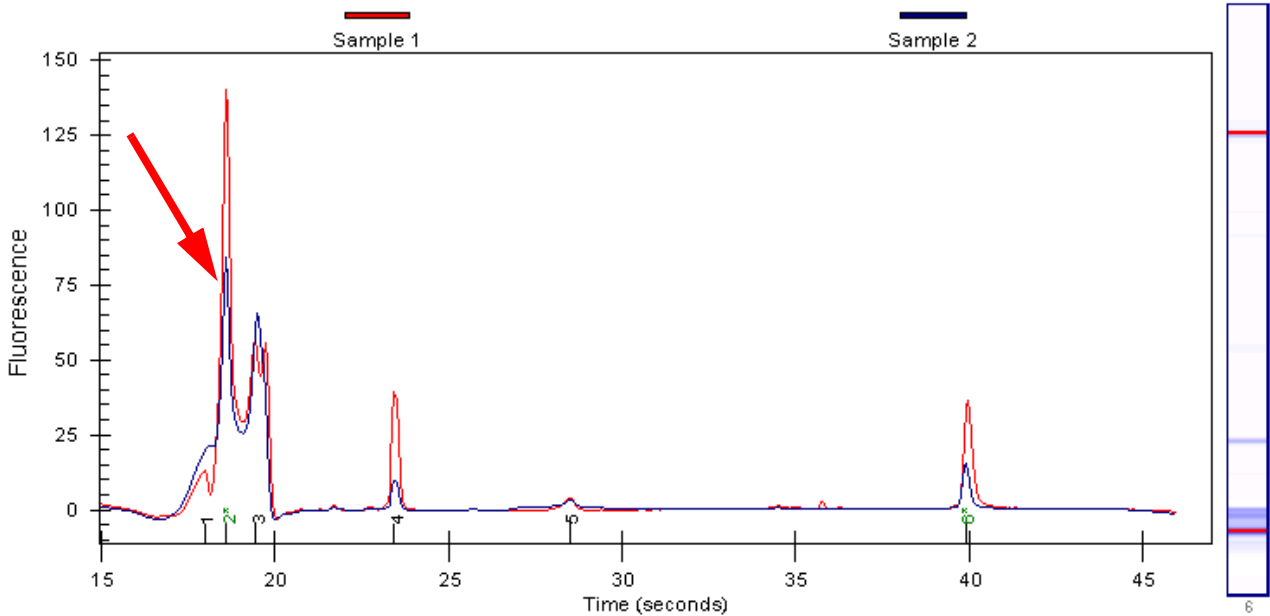
## Low or Missing Upper Marker in Sample

Most Probable Causes	Solution
Sample buffer/denaturing solution not handled according to the instructions.	<p>Refer to the instructions provided with the Reagent Kit guide for storage and preparation of the sample buffer/denaturing solution.</p> <p>To correct for wrong selected upper marker, set upper marker manually. If necessary, adjust peak find settings.</p> <p>For better upper marker identification:</p> <p>Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker.</p>
Incompatible sample component. Some components of the buffer, e.g. CHAPS, TFA, etc. interfere with the upper marker and decrease sensitivity.	<p>See Protein Reagent Kit Guide for a list of compatible buffers and buffer compounds.</p> <p>For an updated list please refer to the web-site <a href="http://www.agilent.com/chem/labonachip">www.agilent.com/chem/labonachip</a>.</p> <p>If necessary dilute, dialyze or desalt the sample.</p> <p>It is recommended to dilute the samples 1:2, 1:4, ... with water to find the optimal dilution.</p>
Diluted samples are too old.	Use diluted samples within one day.

Probable Causes	Solution
Samples not denaturated properly.	Use fresh sample aliquot. Heat samples with denaturing solution for 5 min at 100°C
Samples dried out during denaturation.	Samples were denatured in 1.5 mL tubes. Use 0.5 mL tubes for denaturing.
Least Probable Causes	Solution
Upper marker was digested by proteases (cell lysates).	Add protease inhibitor cocktails to cell lysate samples.

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# High Lower Marker Variability



Show me how to solve High Lower Marker Variability

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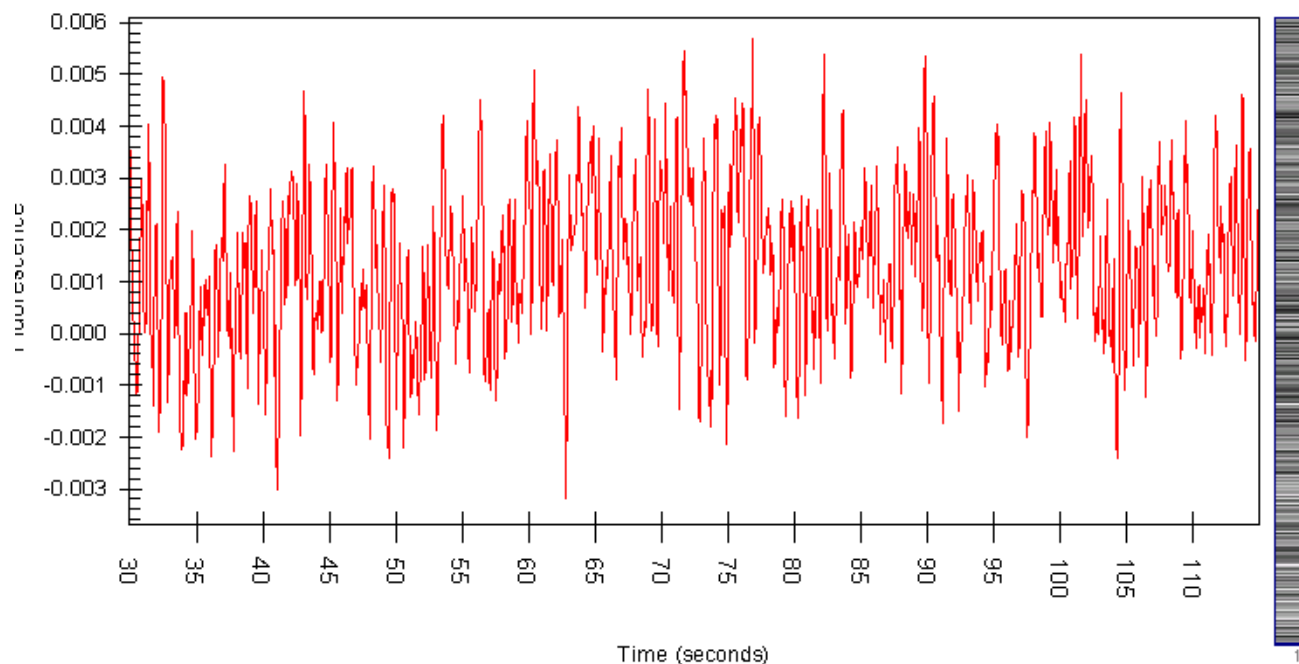
**NOTE** As long as the lower marker is detected, the assay performance is not affected by lower marker variability.

# High Lower Marker Variability

Most Probable Causes	Solution
Buffer components of the sample , e.g. salts, detergents, other additives etc. interfere with the lower marker.	See Protein Reagent Kit Guide for a list of compatible buffers and buffer compounds. If necessary dilute, dialyze or desalt the sample.
Variability of ionic strength of the sample influence the lower marker intensity.	

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# No Peaks



Show me how to solve **No Peaks**

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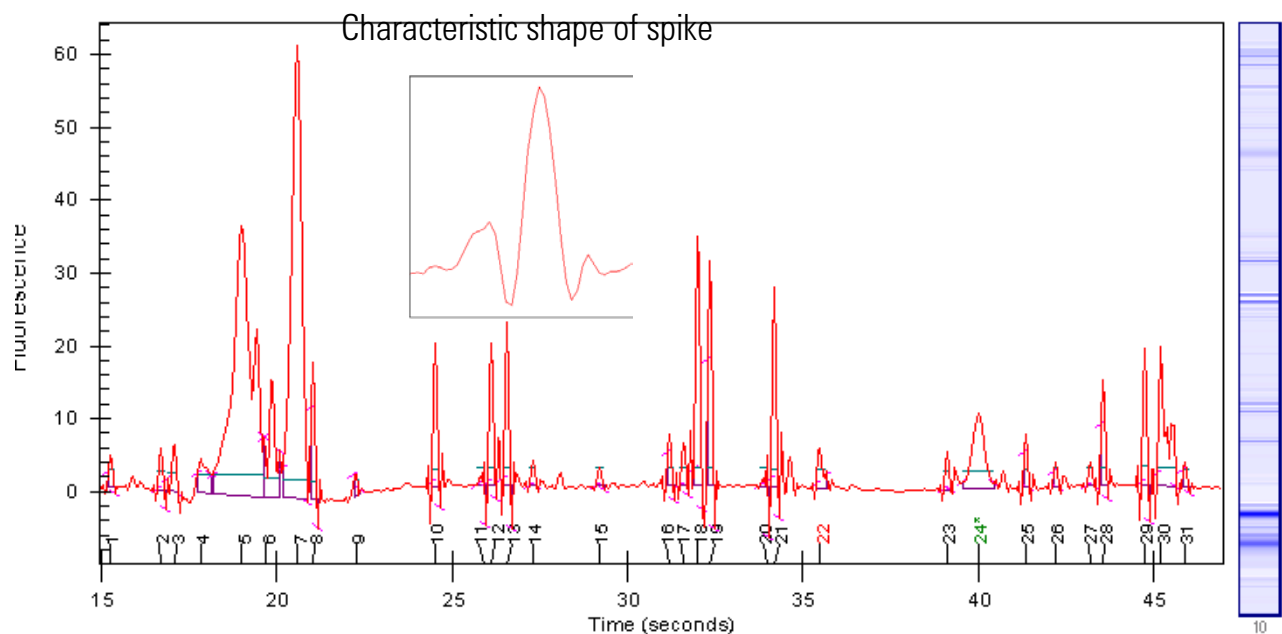
# No Peaks

Most Probable Causes	Solution
Laser defective.	Check laser using the <b>Hardware Diagnostics—48</b> .  If the laser is defective, call Agilent Technologies.
Gel dye mix was loaded in the destain well instead of destaining solution.	Discard chip and prepare new chip according to protocol.
Probable Causes	Solution
Autofocus failure.	Check autofocus using the <b>Hardware Diagnostics—48</b> .  If autofocus fails, call Agilent Technologies.
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> .  If the power supply is defective, call Agilent Technologies.
Fingerprint on focusing lens.	Clean lens like decribed in <b>Lens Maintenance—270</b> .

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# Spikes



Show me how to solve Spikes

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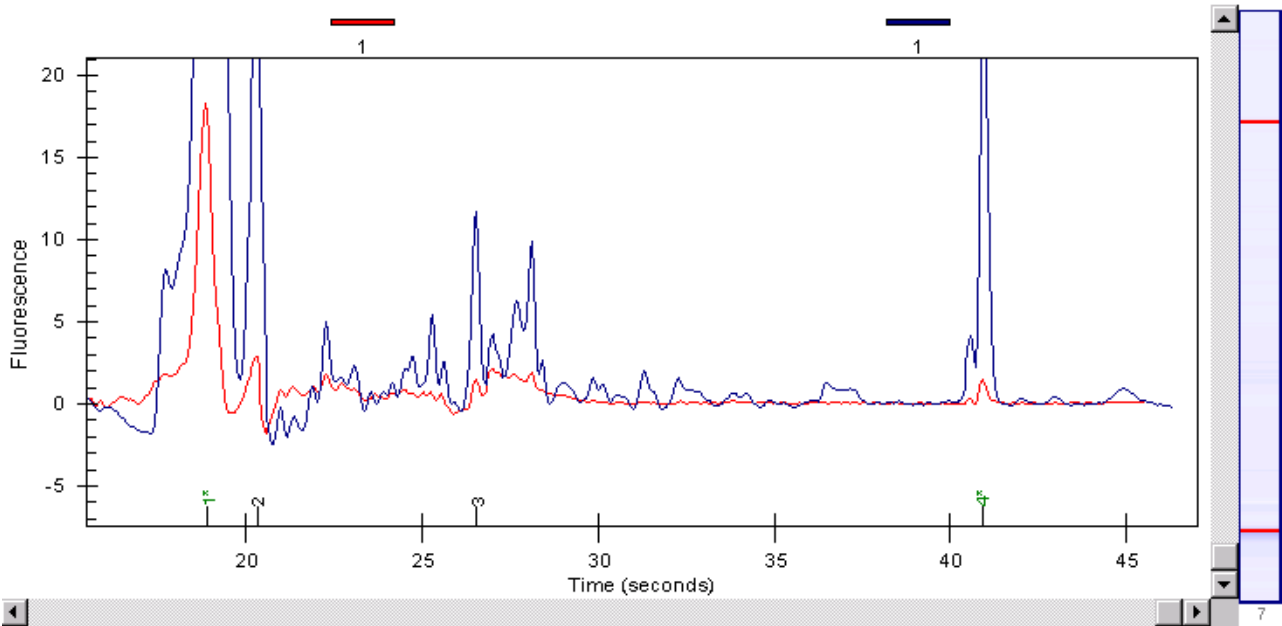
# Spikes

Most Probable Causes	Solution
Chip/gel-dye mix/destaining solution contaminated.	<p>Prepare new chip with new gel-dye mix and new destaining solution:</p> <p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
Gel-dye mix/destaining solution not properly prepared.	<p>Refer to the Reagent Kit Guide for proper preparation of the gel-dye mix and destaining solution. Let the dye warm up to room temperature for 20 min before preparing the gel-dye mix.</p>
Chip not properly prepared.	<p>Prepare a new chip. Allow all reagents and samples to warm up to room temperature before use.</p>

Probable Causes	Solution
Vibration of Agilent 2100 Bioanalyzer.	Don't touch Agilent 2100 Bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Least Probable Causes	Solution
Power outlett	Install power filter.

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# Poor Reproducibility



Show me how to solve Poor Reproducibility

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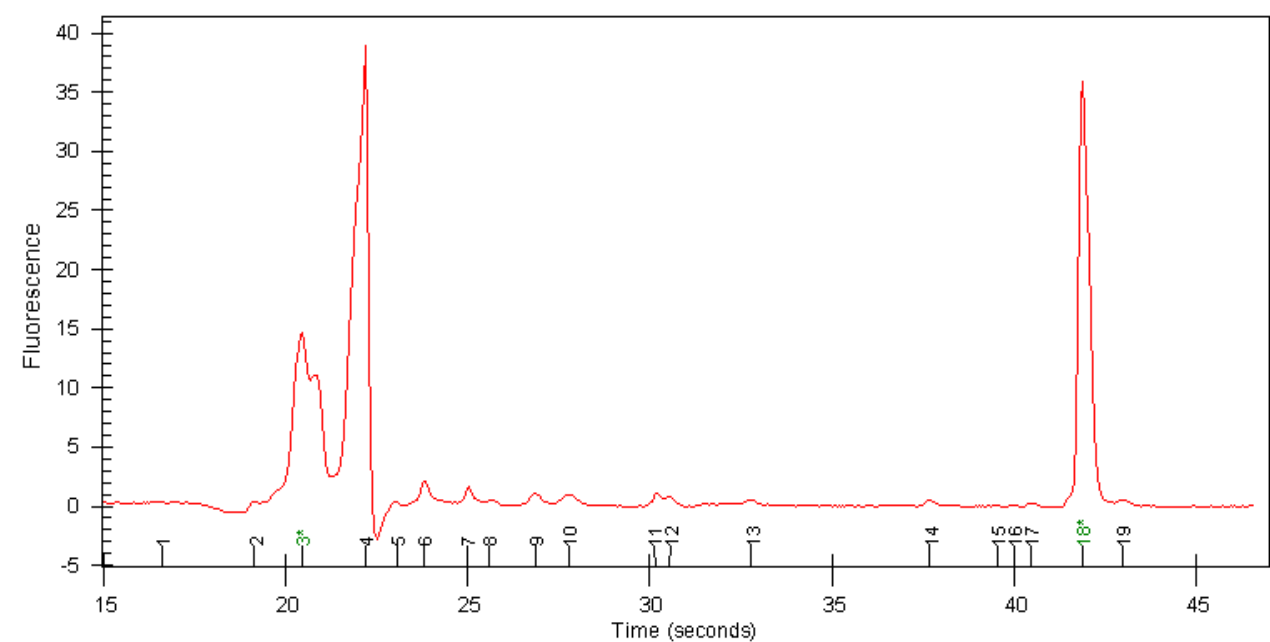
# Poor Reproducibility

Most Probable Causes	Solution
Wrong peak alignment.	<p>Check if alignment is correct (wrong alignment might cause broad peaks compared to the rest of the chip).</p> <p>For better identification of the lower and upper marker:</p> <p>Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the lower and upper marker.</p>
One Sample not denaturated properly.	Use fresh sample aliquot. Heat samples with denaturing solution for 5 min at 100°C
One Sample dried out during denaturation.	Samples were denaturated in 1.5 mL tubes. Use 0.5 mL tubes for denaturing.
Reducing agent (BME or DTT) was added in one sample and not in the other.	Refer to the Reagent Kit Guide for proper sample reduction.

Probable Causes	Solution
Diluted samples are too old.	Use diluted samples within one day.
Buffer component interferes with LDS/SDS in sample buffer.	<p>See Protein Reagent Kit Guide for a list of compatible buffers and buffer compounds.</p> <p>For an updated list please refer to the web-site <a href="http://www.agilent.com/chem/labonachip">www.agilent.com/chem/labonachip</a>.</p> <p>If necessary dilute, dialyze or desalt the sample.</p>

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# No or Low Sample Peaks



Show me how to solve No or Low Sample Peaks

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## No or Low Sample Peaks

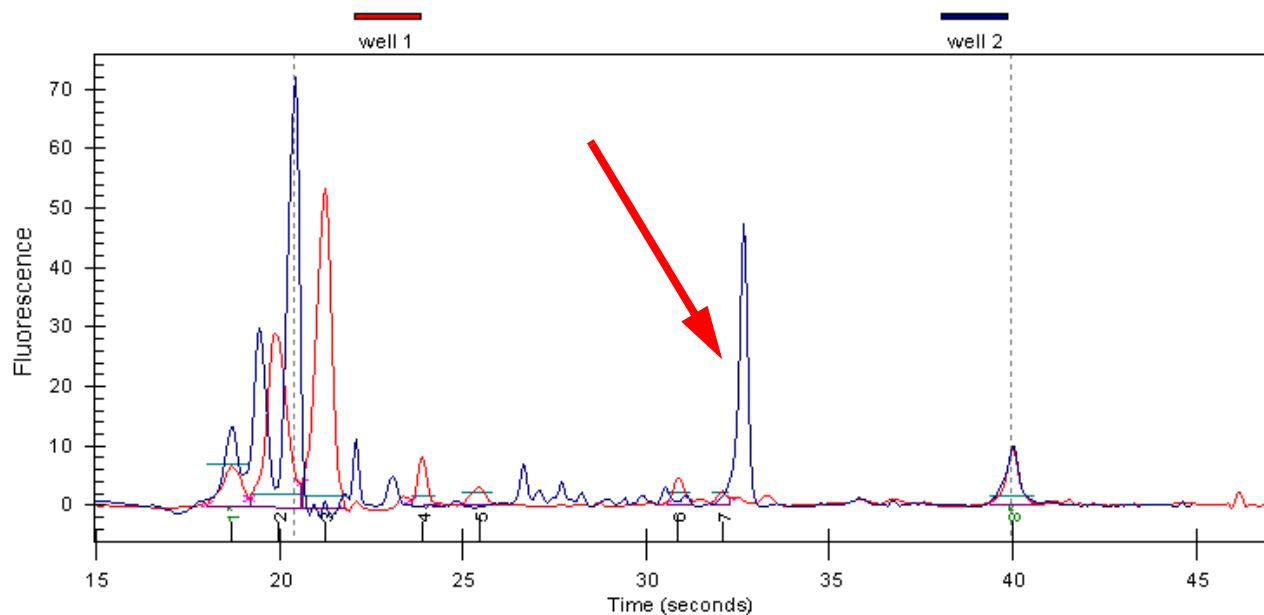
Most Probable Causes	Solution
Protein concentration in samples too low.	Use protein concentration according to specifications given in the Reagent Kit Guide.
Too high salt concentration in samples.	Sensitivity is strongly affected by salt concentration. Dilute samples in deionized H <sub>2</sub> O, dialyze samples against low salt buffer or desalt samples using spin filters.
SDS not completely dissolved in dye concentrate.	Let dye concentrate equilibrate to room temperature for 20 min before use. Check for undissolved SDS crystals in the tube. Vortex dye concentrate well before use. If necessary heat the sample buffer to 37°C for 2 min.
Probable Causes	Solution
Samples not completely denatured.	Use fresh sample aliquot. Heat sample/denaturing solution for 5 min at 100°C.
Sample/denaturing solution are dried out.	Sample/denaturing solution were denatured in 1.5 mL tubes. Use 0.5 mL tubes for denaturing.
Pipetting error during preparation of mixtures.	Check dilution procedure. Check calibration of pipette.
Diluted samples are too old.	Use diluted samples within one day.



The gel dye mix was not replaced after priming the chip.	Prepare new chip according to the Protein 200 Reagent Kit Guide.
Least Probable Causes	Solution
Samples dissolved in acidic buffer.	Neutralize samples with appropriate buffer or dilute samples in deionized H <sub>2</sub> O. Alternatively dialyze samples against buffer with medium pH.
Autofocus failure.	Check autofocus by means of the <b>Hardware Diagnostics—48</b> .  If autofocus fails, call Agilent Technologies.

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## Apparently Missing Sample Peak



Show me how to solve **Apparently Missing Sample Peak**

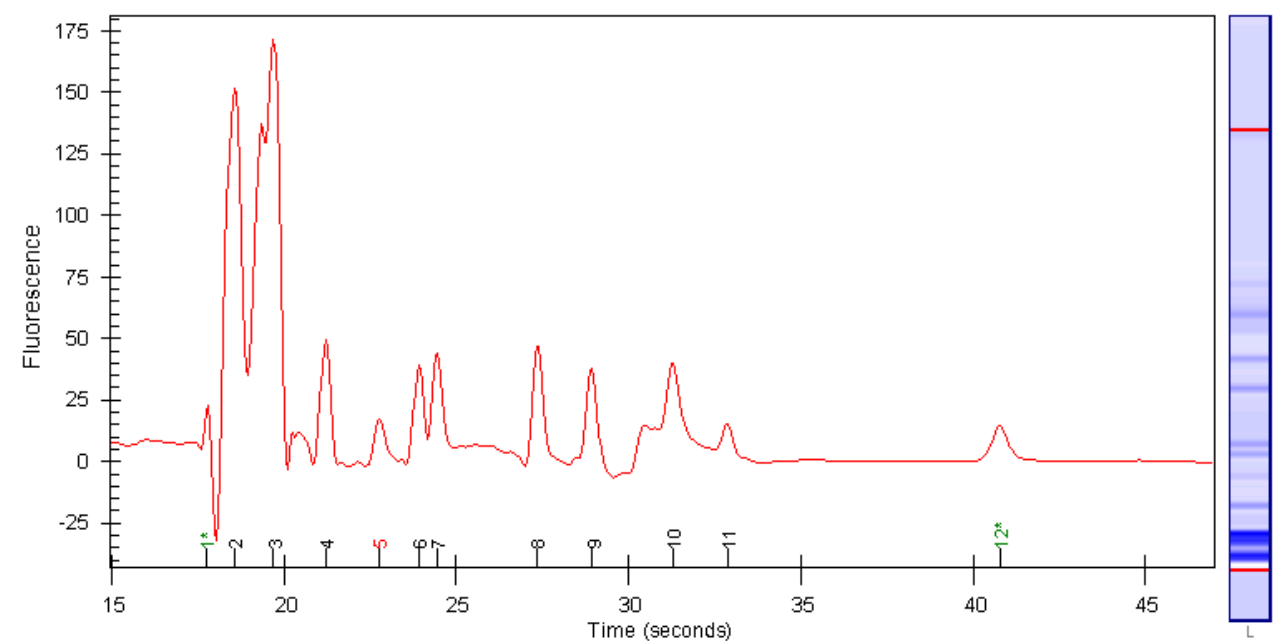
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# Apparently Missing Sample Peak

Most Probable Causes	Solution
Wrongly assigned upper marker.	Refer to the instructions provided with the Reagent Kit guide for storage and preparation of the sample buffer/denaturing solution.
Sample buffer/denaturing solution not handled according to the instructions.	To correct for wrong selected upper marker, set upper marker manually. If necessary, adjust peak find settings.
Because of low intensity, the software identified sample peak as upper marker.	For better upper marker identification:  Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker.

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# Low Ladder Peaks



Show me how to solve **Low Ladder Peaks**

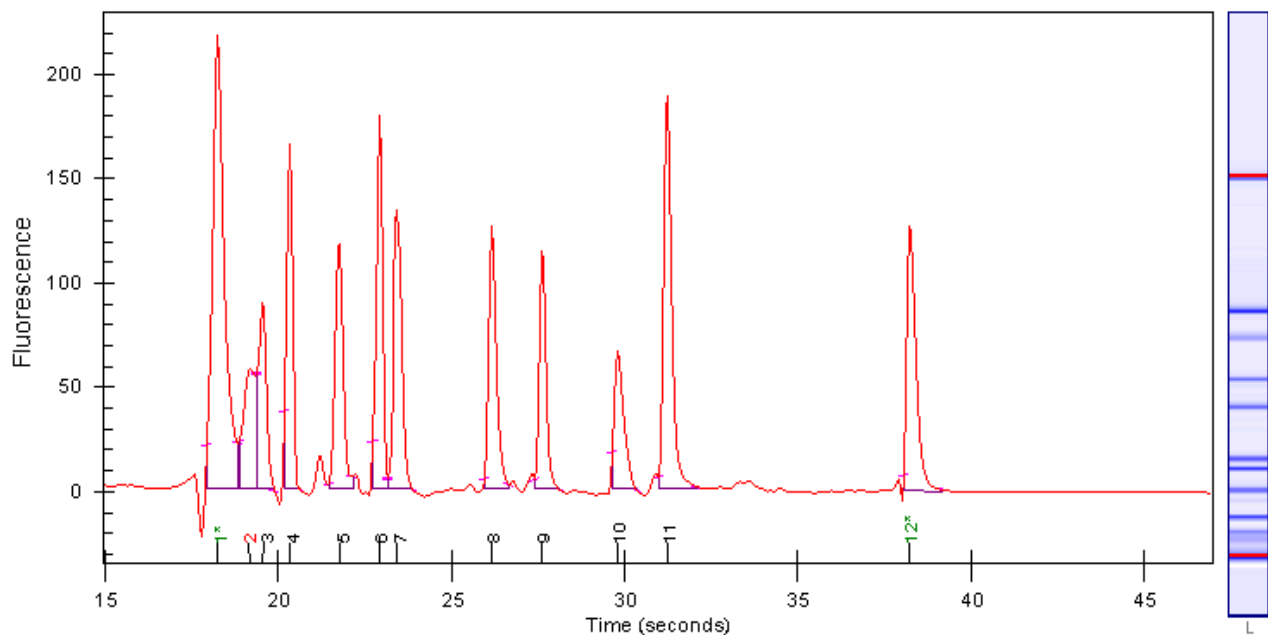
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# Low Ladder Peaks

Most Probable Causes	Solution
Ladder degraded.	Refer to the Protein Reagent Kit Guide for proper ladder storage.  Optional: Prepare ladder aliquots.  Use a new aliquot.
Ladder not diluted after denaturation.	Refer to the Ragent Kit Guide for proper chip preparation.
Probable Causes	Solution
Ladder not completely denaturated.	Use fresh ladder aliquot. Heat ladder for 5 min at 100°C.
Ladder dried out.	Ladder was denaturated in 1.5 mL tubes. Use 0.5 mL tubes for denaturating.
Diluted ladder is too old.	Use diluted ladder within one day.
Pipetting error during preparation of mixtures.	Check dilution procedure.  Check calibration of pipette.

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## Wrong Alignment of Ladder Peaks



Show me how to solve **Wrong Alignment of Ladder Peaks**

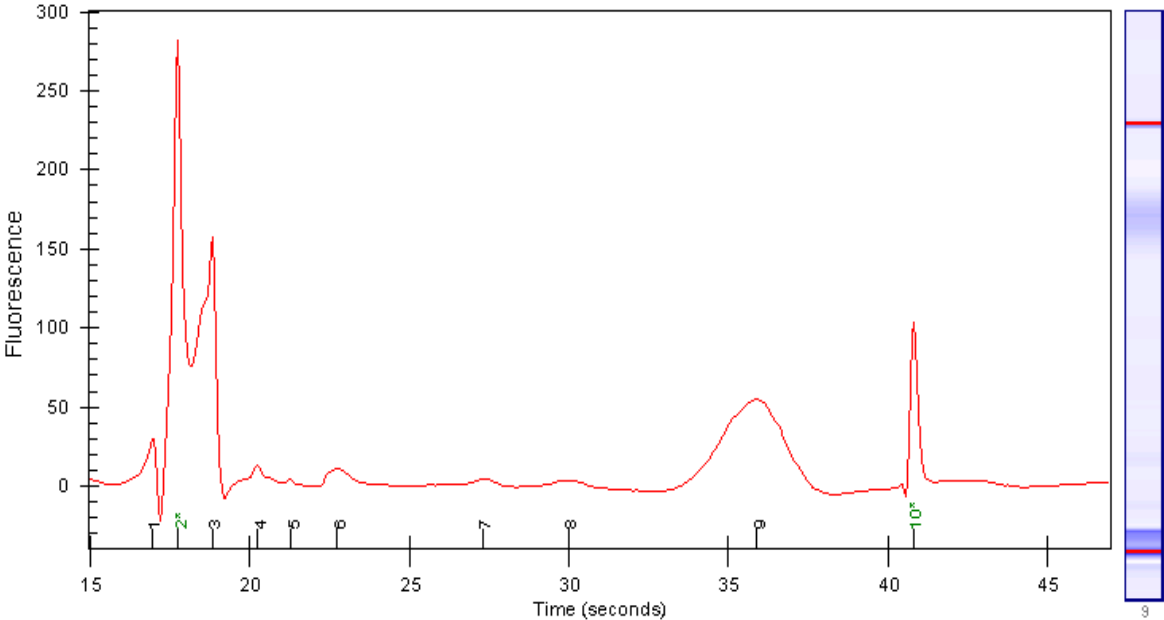
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# Wrong Alignment of Ladder Peaks

Most Probable Causes	Solution
Low intensity of upper marker. The software identifies ladder peak as upper marker.	<p>If necessary adjust peak find settings and exclude low intensity peaks.</p> <p>See</p> <p><b>Low Ladder Peaks—221</b></p> <p>for probable causes.</p> <p>For better upper marker identification:</p> <p>Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker.</p>

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# Broad Peaks



Show me how to solve **Broad Peaks**

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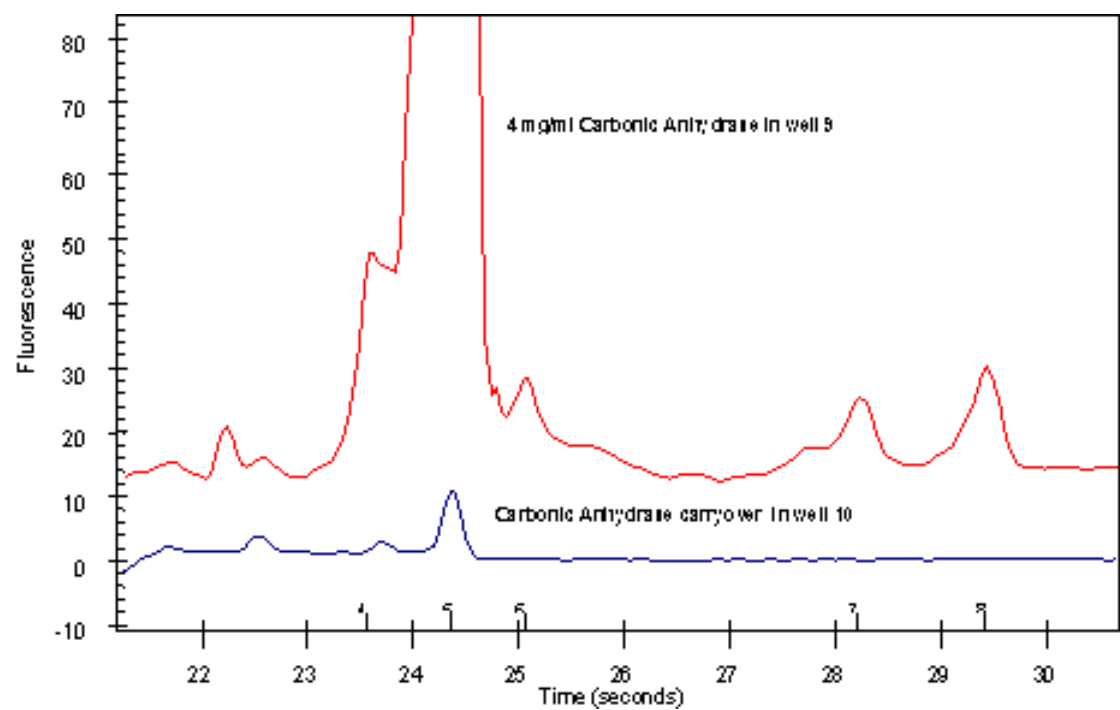
# Broad Peaks

Most Probable Causes	Solution
Wrong peak alignment.	<p>Check if alignment is correct (wrong alignment might cause broad peaks compared to the rest of the chip).</p> <p>For better identification of the lower and upper marker:</p> <p>Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the lower and upper marker.</p>
Chip not properly primed. Air bubble in chip.	<p>Use a new chip. Check chip priming station/syringe for good seal (see <b>Maintaining the Chip Priming Station—271</b>).</p> <p>Check if clip and base plate of priming station are in the right position (see Protein Reagent Kit Guide).</p>
Leak Current due to contaminated electrodes. Chip was left in instrument after run.	<p>Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b>. Don't leave chip in instrument after run. Clean electrodes after each run.</p> <p>Replace electrode cartridge.</p>

Probable Causes	Solution
Sample was not denaturated properly.	Use fresh sample aliquot. Heat sample/ denaturing solution for 5 min at 100°C.
Reducing agent (BME or DTT) was added in one sample and not in the other.	Refer to the Reagent Kit Guide for proper sample reduction.
High voltage power supply defective.	Check high voltage power supply using the <b>Hardware Diagnostics—48</b> .  If the power supply is defective, call Agilent Technologies.

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# Cross Contamination



Show me how to solve **Cross Contamination**

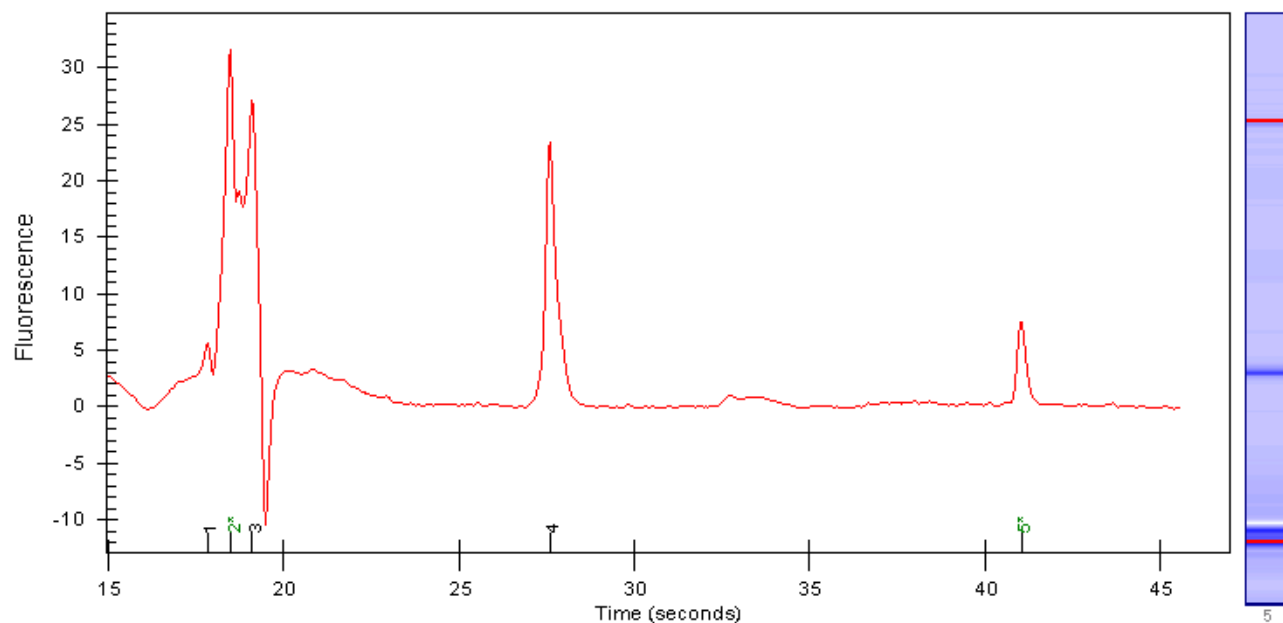
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# Cross Contamination

Most Probable Causes	Solution
Sample concentration too high.	Use sample concentration according to the Protein Reagent Kit Guide.
Contaminated electrodes. Chip left in instrument after run.	Clean electrodes with analysis-grade water and a toothbrush, see <b>Maintenance—246</b> .  Don't leave chip in instrument after run. Clean electrodes after each run.
Probable Causes	Solution
Pipetting error during preparation of mixtures.	Check dilution procedure.  Check calibration of pipette.
Chip pipetting error.	Use new chip and pipette again.  Use appropriate pipette and tips.

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# Dips



## NOTE

As long as the lower marker is detected, the assay performance is not affected by dips.

Show me how to solve **Dips**

Back to **Symptoms**

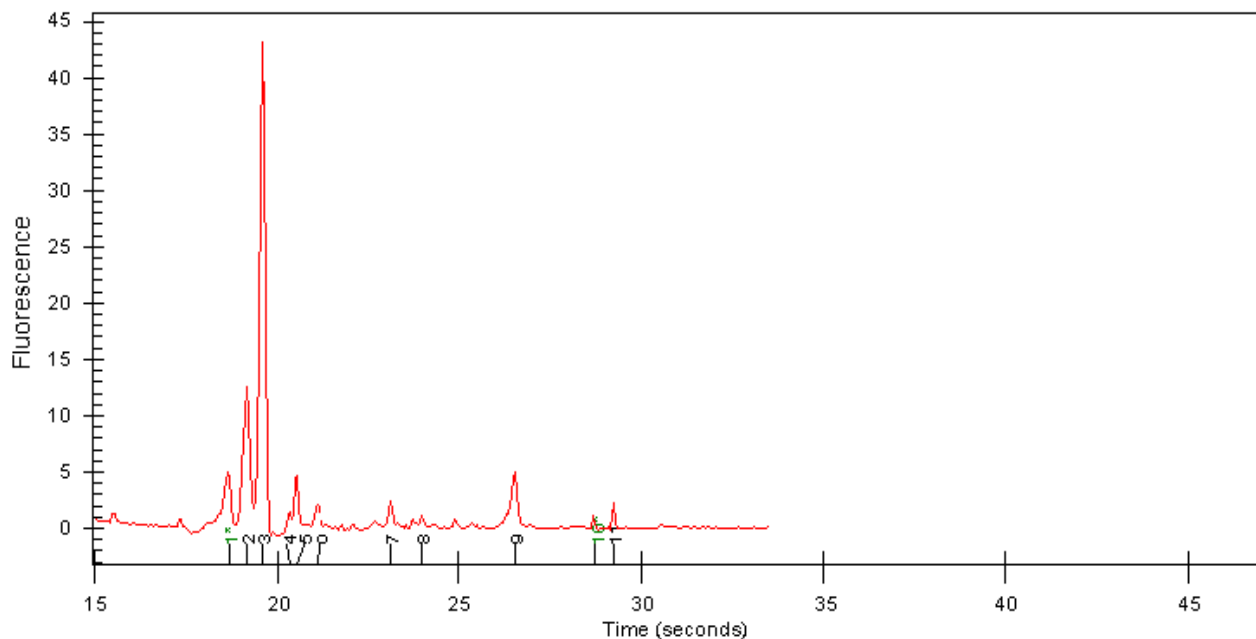
# Dips

Most Probable Causes	Solution
Sample contains additional detergents and/or dyes.	See Protein Reagent Kit Guide for a list of compatible buffers and buffer compounds.  For an updated list please refer to the web-site <a href="http://www.agilent.com/chem/labonachip">www.agilent.com/chem/labonachip</a> .  If necessary dilute, dialyze or desalt the sample.

Back to **Symptoms**

# List of Protein Electropherograms

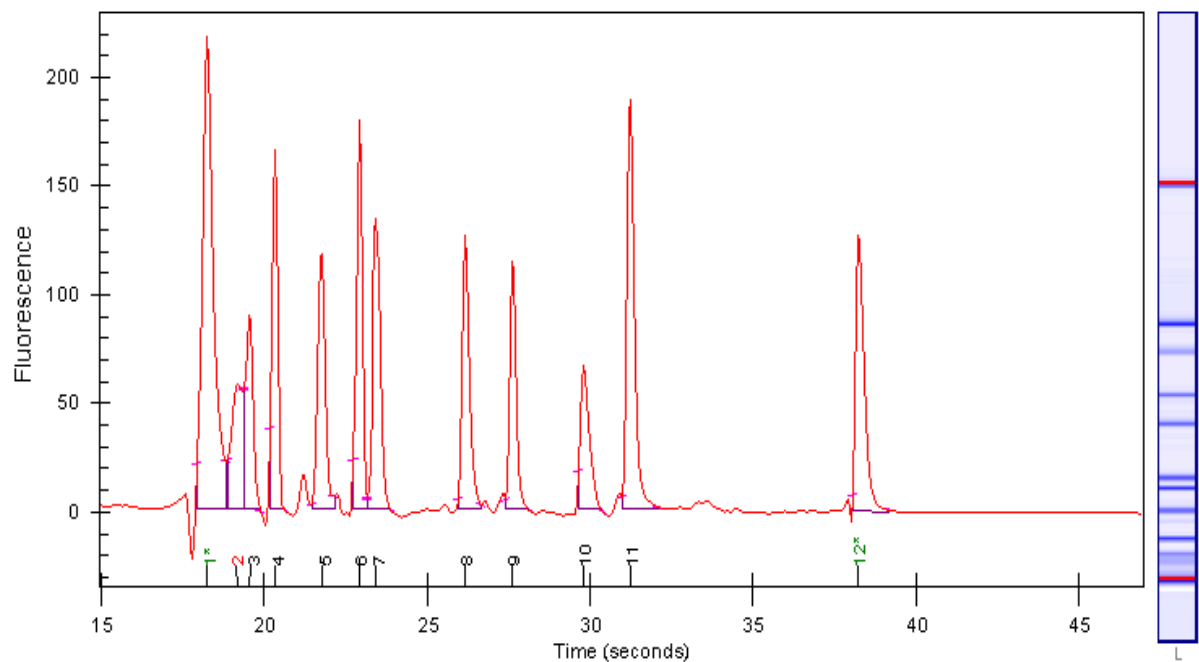
## Apparently Short Run Time



Show me how to solve **Apparently Short Run Time**

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Additional Sample or Ladder Peaks

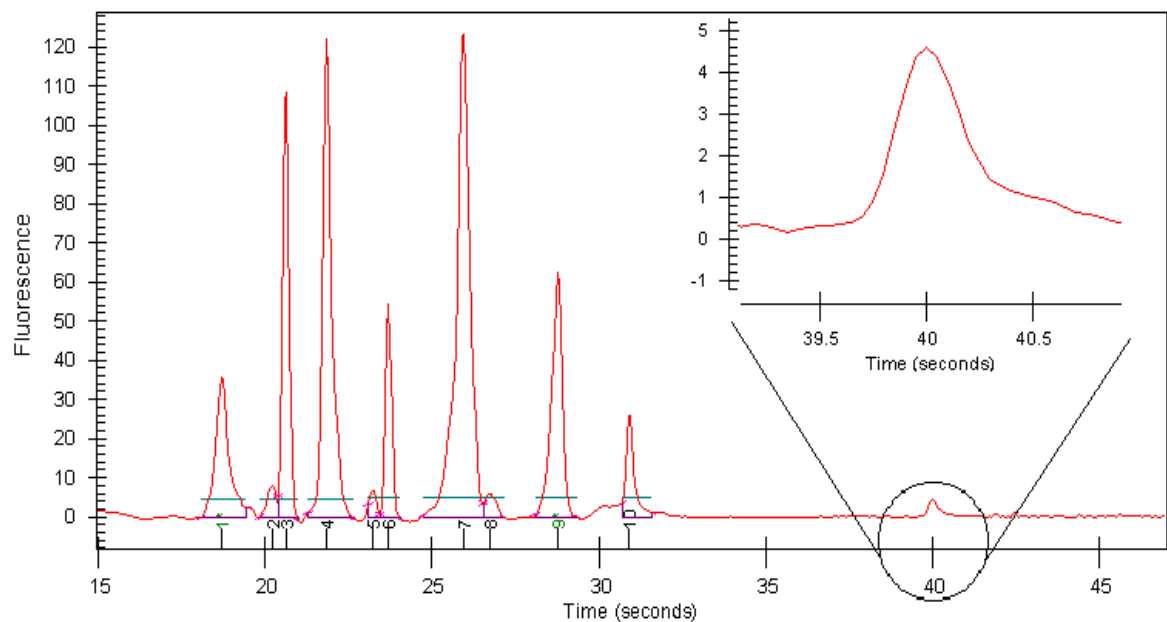


Show me how to solve Additional Sample or Ladder Peaks

Back to the Top of List



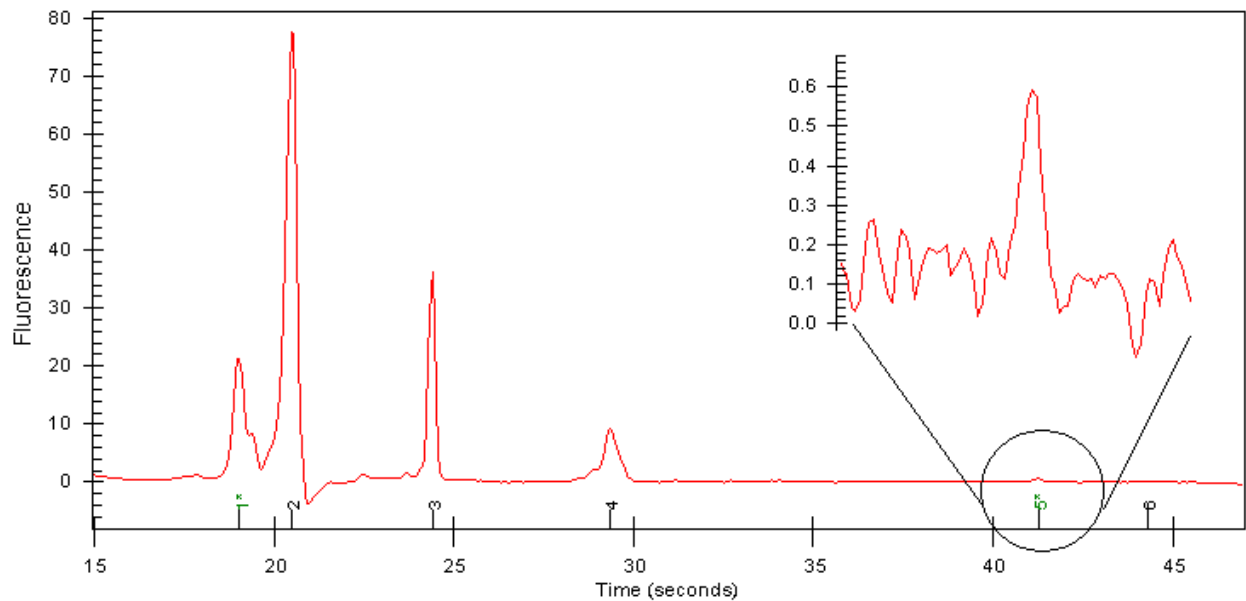
# Low or Missing Upper Marker in Ladder



Show me how to solve **Low or Missing Upper Marker in Ladder**

Back to the **Top of List**

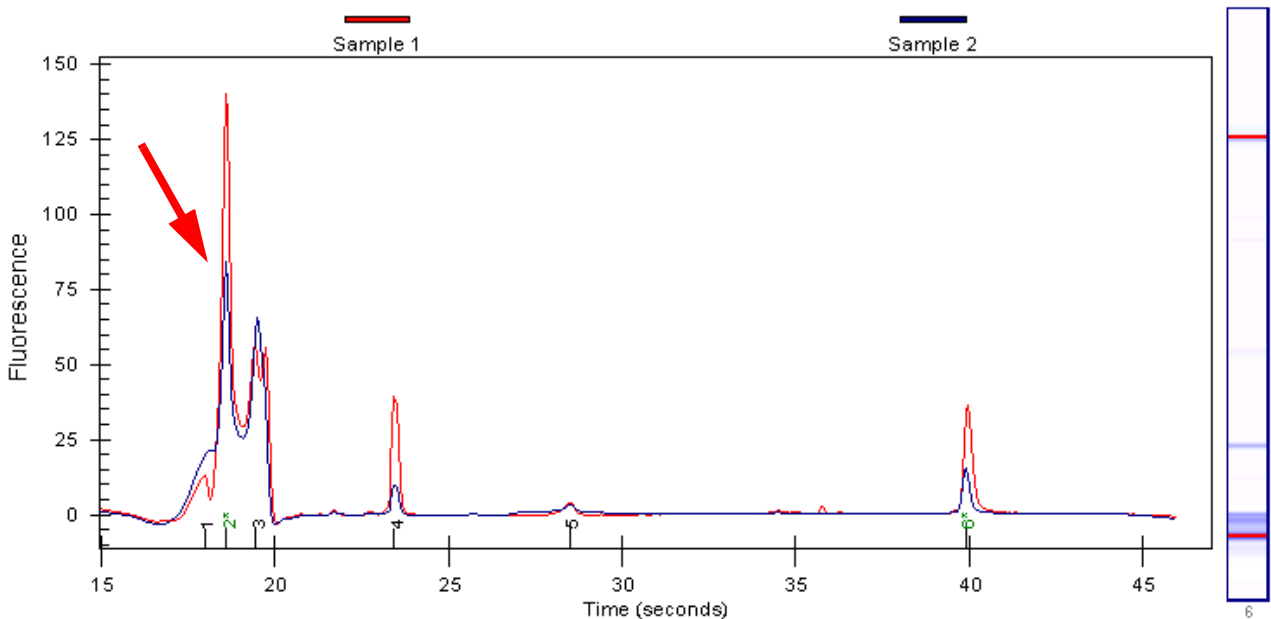
Low or Missing Upper Marker in Sample



Show me how to solve **Low or Missing Upper Marker in Sample**

Back to the **Top of List**

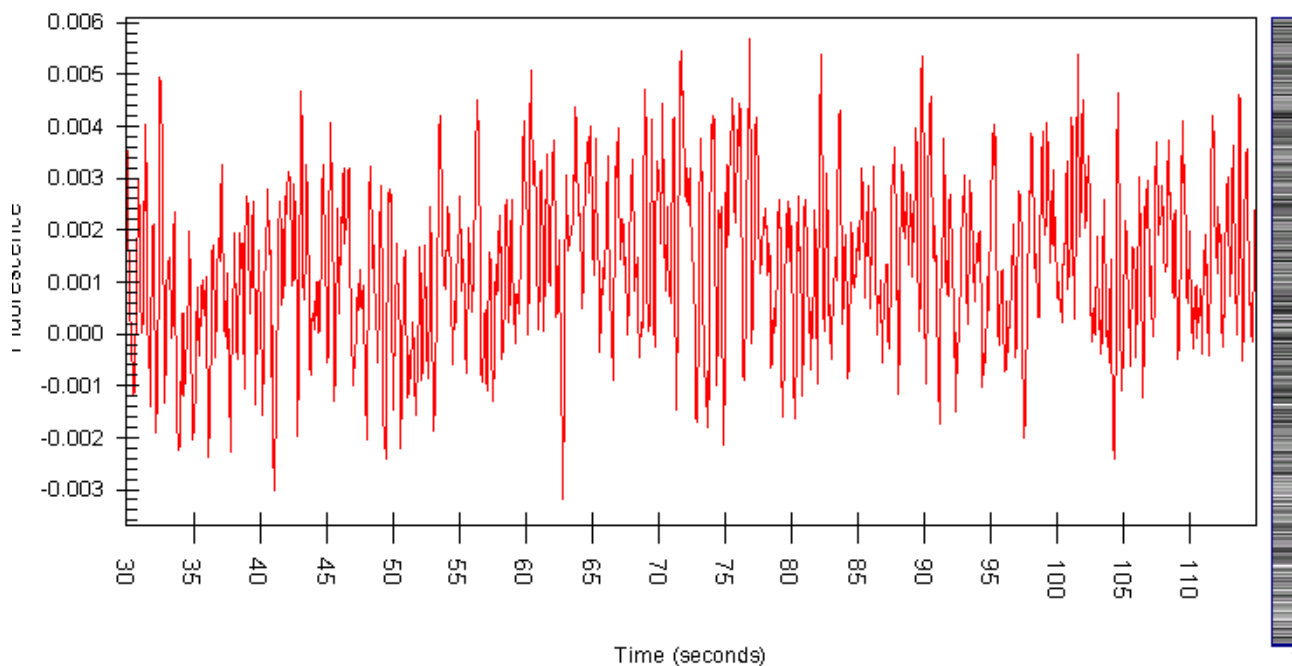
# High Lower Marker Variability



Show me how to solve **High Lower Marker Variability**

Back to the **Top of List**

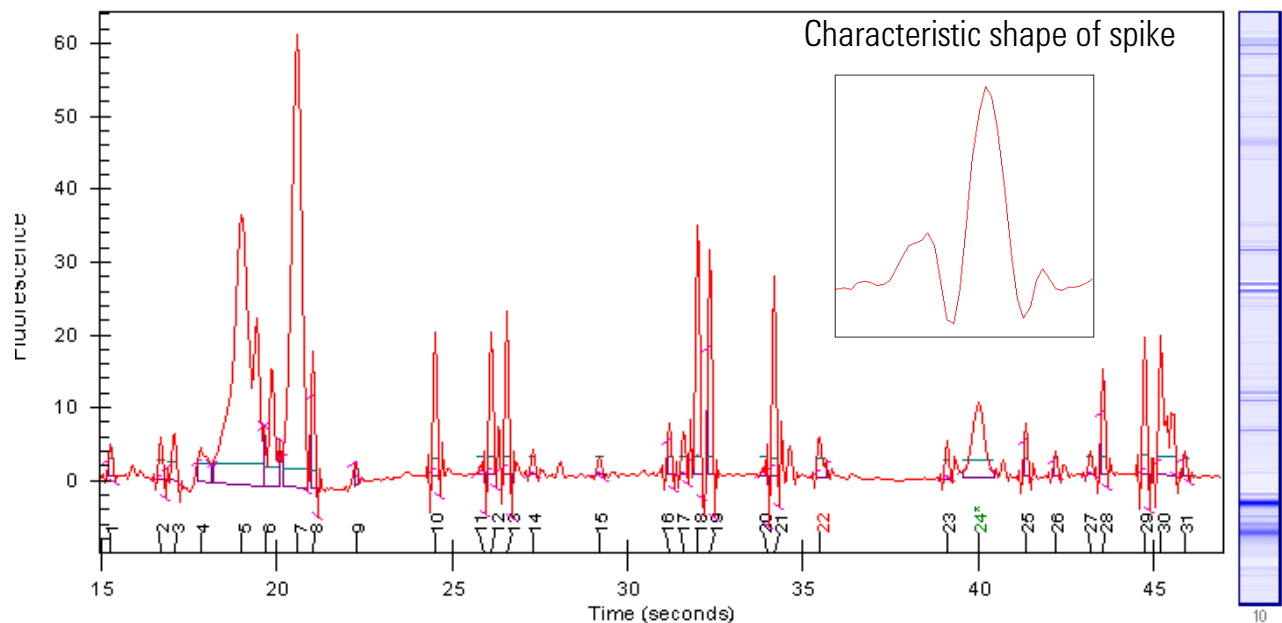
## No Peaks



Show me how to solve **No Peaks**

Back to the **Top of List**

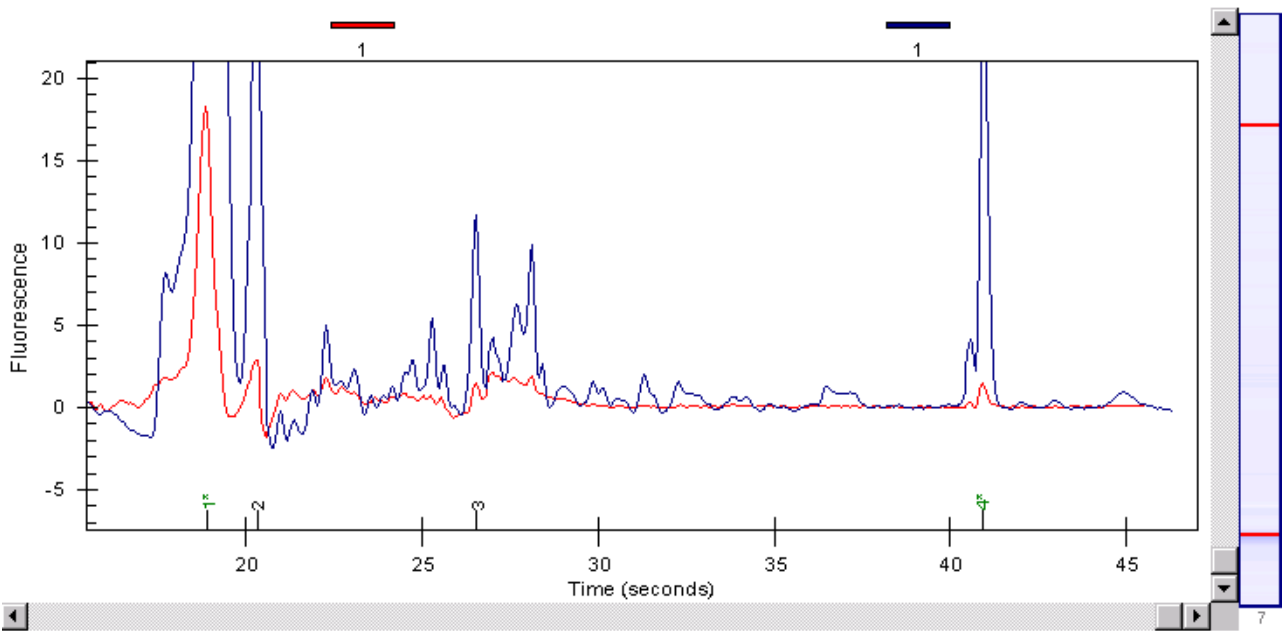
# Spikes



Show me how to solve Spikes

Back to the **Top of List**

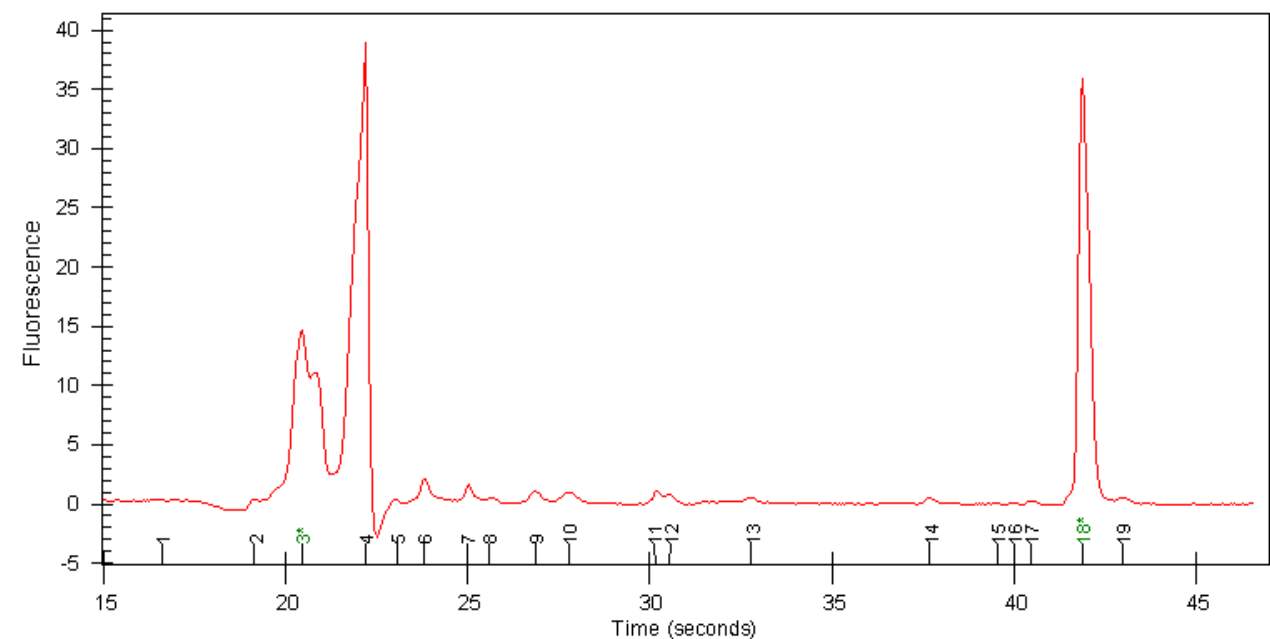
Poor Reproducibility



Show me how to solve Poor Reproducibility

Back to the **Top of List**

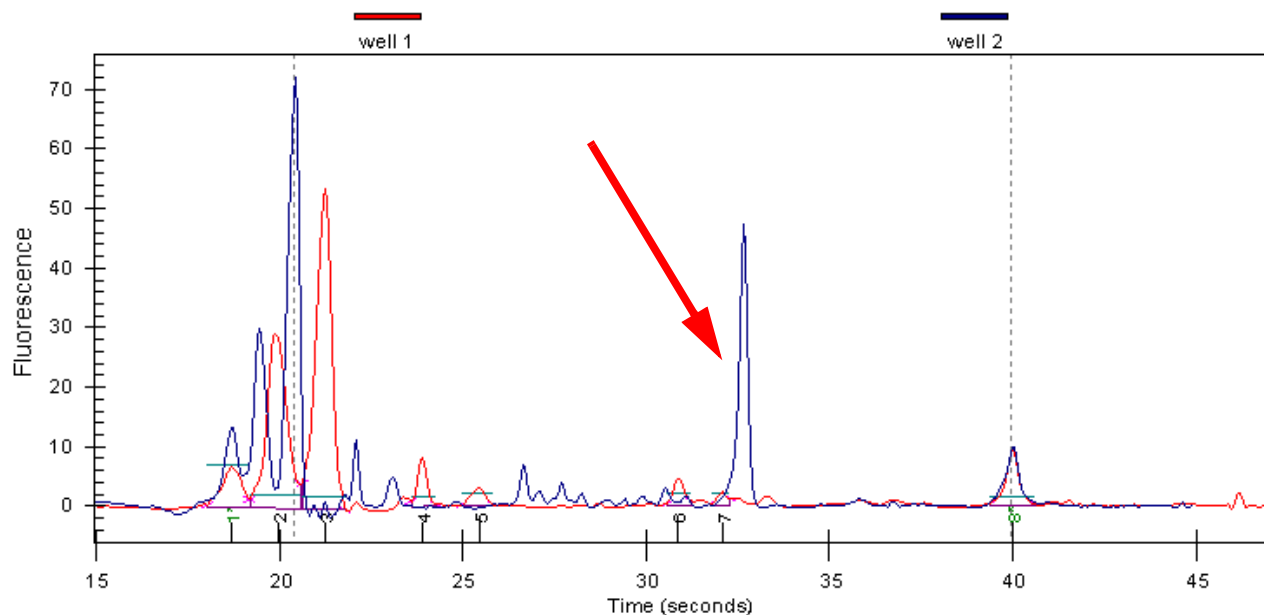
# No or Low Sample Peaks



Show me how to solve No or Low Sample Peaks

Back to the **Top of List**

## Apparently Missing Sample Peak

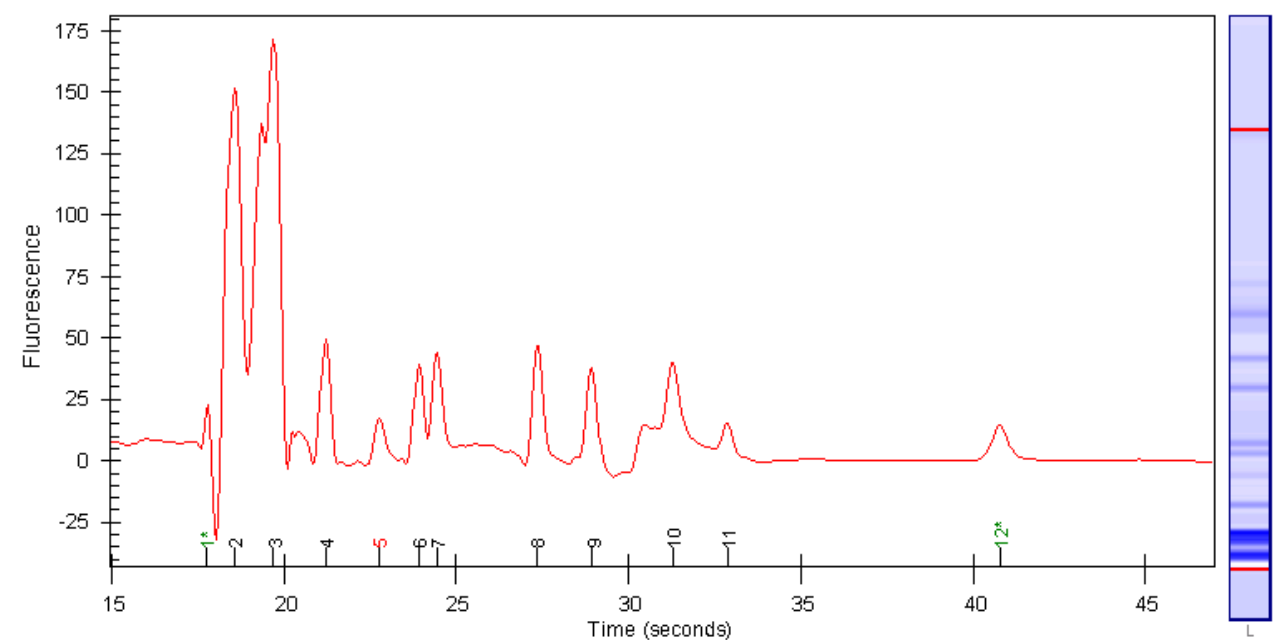


Show me how to solve **Apparently Missing Sample Peak**

[Back to the Top of List](#)



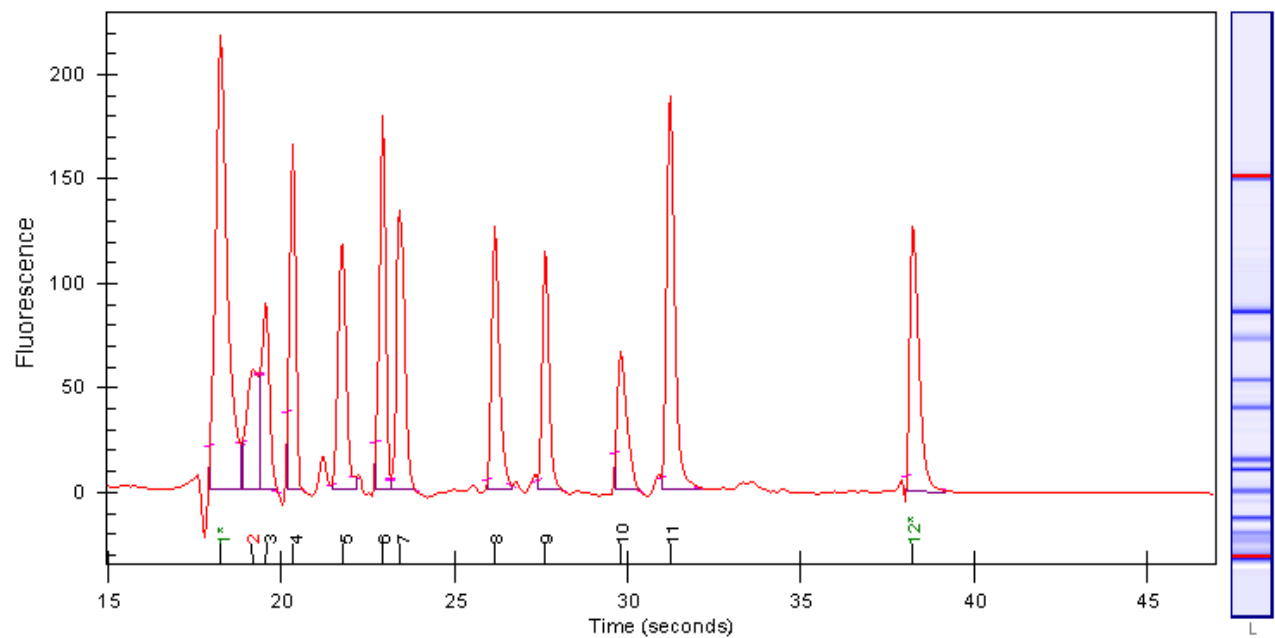
# Low Ladder Peaks



Show me how to solve **Low Ladder Peaks**

Back to the **Top of List**

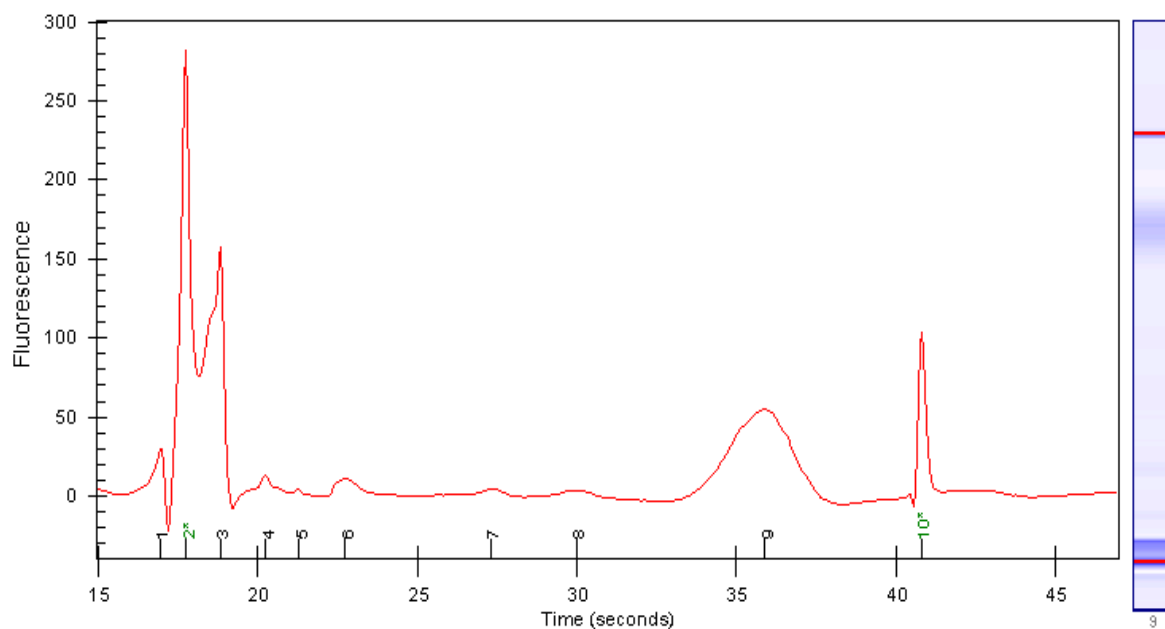
# Wrong Alignment of Ladder Peaks



Show me how to solve Wrong Alignment of Ladder Peaks

Back to the **Top of List**

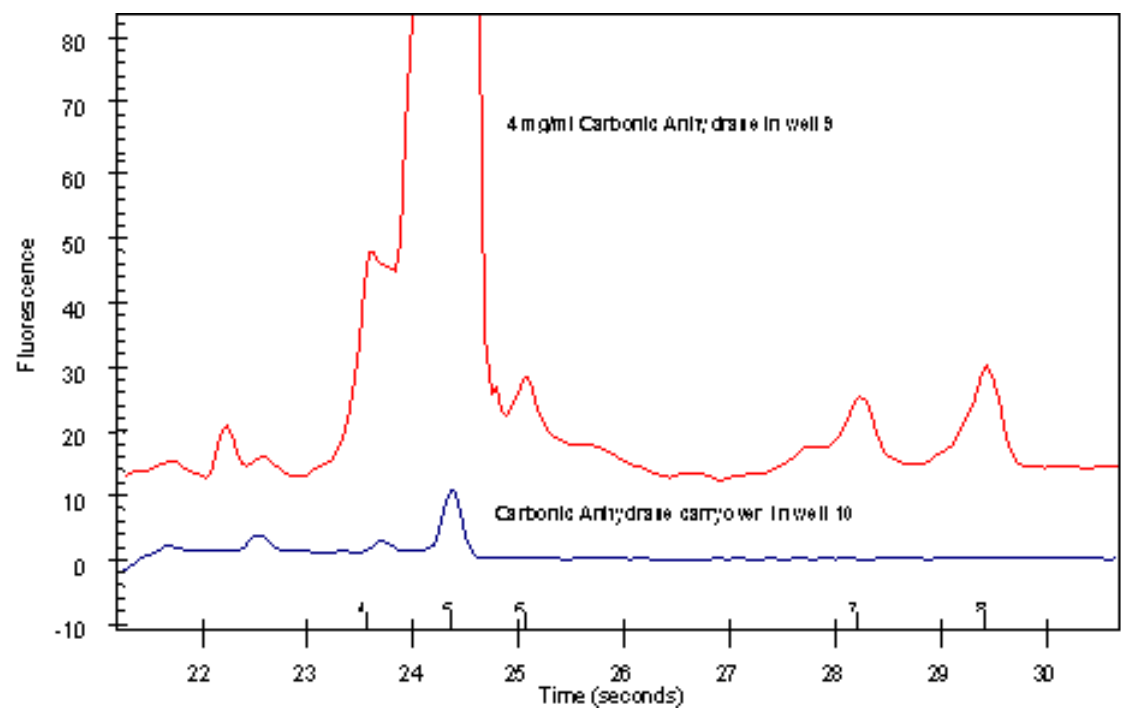
## Broad Peaks



Show me how to solve **Broad Peaks**

Back to the **Top of List**

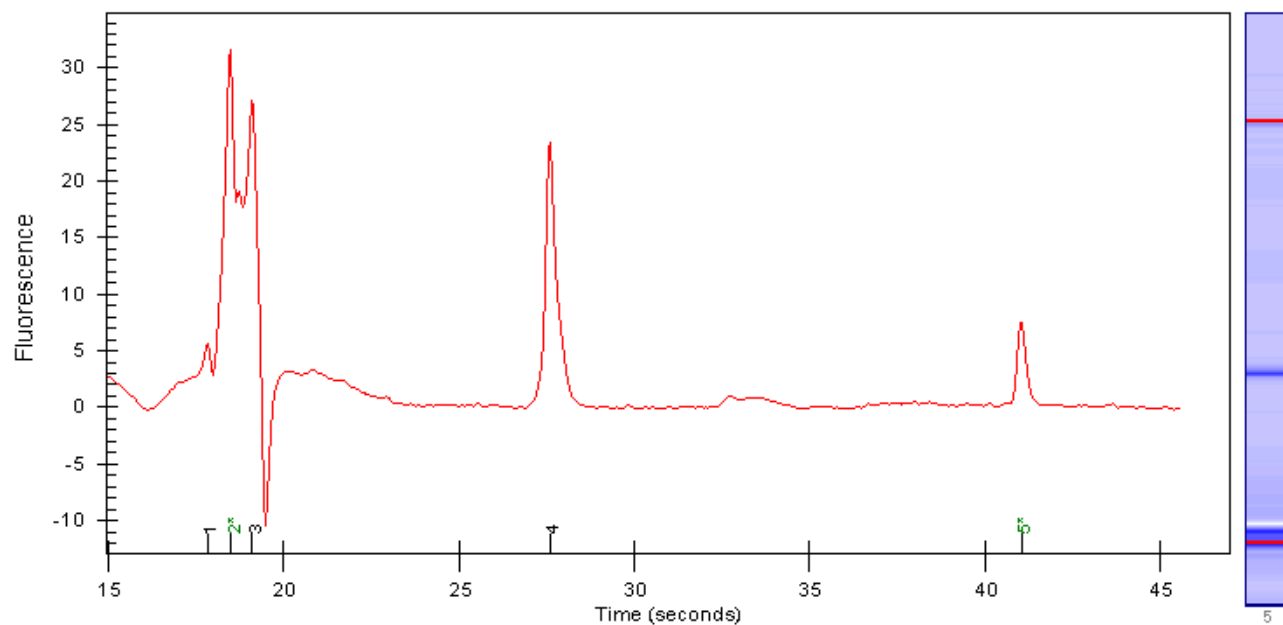
# Cross Contamination



Show me how to solve **Cross Contamination**

Back to the **Top of List**

## Dips



Show me how to solve **Dips**

Back to the **Top of List**

# Maintenance

## Care and Cleaning of the Agilent 2100 Bioanalyzer

The Agilent 2100 bioanalyzer should be kept clean. Cleaning should be done with a damp lint-free cloth. Do not use an excessively damp cloth allowing liquid to drip into the Agilent 2100 bioanalyzer.

---

**WARNING** Do not let liquid drip into the Agilent 2100 bioanalyzer. It could cause a shock or it could damage the Agilent 2100 bioanalyzer.

---

- 1 Open the lid.
- 2 Take out the electrode or the pressure cartridge
- 3 If any solutions have dried on the electrodes or pressure adapter, use a damp lint-free cloth to clean the electrodes or adapter.

---

**WARNING** When working with solvents please observe appropriate safety procedures (for example goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.

---

## **WARNING**

---

**If pathogenic, toxic, or radioactive samples are intended to be used in this instrument, it is the responsibility of the user to ensure that all necessary safety regulations, guidelines, precautions and practices are adhered to accordingly. Ask your laboratory safety officer to advise you about the level of containment required for your application and about proper decontamination or sterilization procedures to follow if fluids escape from containers.**

---

# Electrode Cartridge Maintenance

## Daily Basis—Electrode Cleaner

To avoid cross contamination due to contaminated electrode tips, clean electrodes after each run.

- 1 Slowly fill one of the wells of the the electrode cleaner with 350  $\mu$ l deionized analysis-grade water.

---

**NOTE** Never fill too much water in the electrode cleaner. This could cause liquid spill or contamination of the electrodes.

---

- 2 Open the lid and place electrode cleaner in the Agilent 2100 Bioanalyzer.
- 3 Close the lid and leave it closed for about 5 seconds.
- 4 Open the lid and remove the electrode cleaner.
- 5 Wait another 10 seconds for the water on the electrodes to evaporate.

Depending on the sensitivity of your measurements and the adhesive forces of your sample, you have to change the water in the electrode cleaner after each use.

---

**WARNING** Never use a cloth to clean the electrodes. Electrostatic discharge might damage the high voltage power supplies.

---

## Daily Basis—For RNA Assay Only

To avoid decomposition of your RNA sample, follow this electrode decontamination procedure on a daily basis.



- 1** Slowly fill one of the wells of an electrode cleaner with 350  $\mu$ l RNaseZAP.
- 2** Open the lid and place electrode cleaner in the Agilent 2100 Bioanalyzer.
- 3** Close the lid and leave it closed for about 1 minute.
- 4** Open the lid and remove the electrode cleaner—label the electrode cleaner and keep for future use. You can reuse the electrode cleaner for all the chips in the kit. If the electrode cleaner dries out, simply refill.
- 5** Slowly fill one of the wells of *another* electrode cleaner with 350  $\mu$ l RNase-free water.
- 6** Place electrode cleaner in the Agilent 2100 Bioanalyzer.
- 7** Close the lid and leave it closed for about 10 seconds.
- 8** Open the lid and remove the electrode cleaner.

Wait another 10 seconds for the water on the electrodes to evaporate.

## **Every 3 Months**

Evaporation of liquid from the chip could cause salt to coat the electrodes and the area between the electrodes. Leak currents, which distort the measurement results, could result.

Remove electrode cartridge from the Agilent 2100 Bioanalyzer. For changing the cartridge, please select your cartridge type:

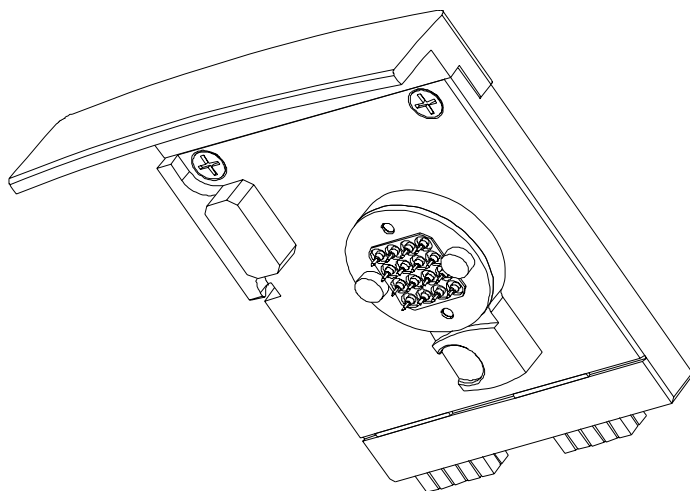
Changing the 16-Pin Cartridge—251

Changing the 16-Pin Bajonet Cartridge—257

## Changing the 16-Pin Cartridge

The 16-pin cartridge (reorder number 5064-8244) contains 16 electrodes configured to fit in the wells of a LabChip. The electrodes make contact with the liquid in the wells when the lid of the Agilent Technologies 2100 bioanalyzer is closed, see Figure 1. The cartridge can be removed if the electrodes become contaminated or damaged.

Figure 1      16-Pin Cartridge



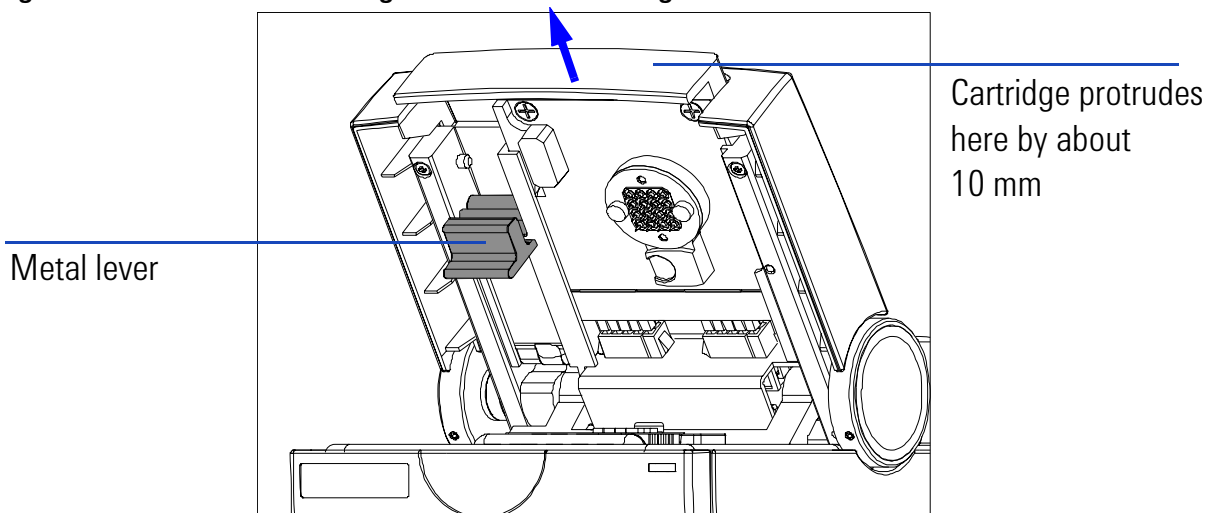
# Removing the 16-Pin Cartridge

**WARNING** Do not touch the electrodes while the cartridge is in the 2100 bioanalyzer—the electrodes and high voltage power supplies can be easily damaged.

- 1 Turn off line power to the 2100 bioanalyzer. The line switch is located at the rear of the 2100 bioanalyzer.
- 2 Open the lid. Move the metal lever on the left of the inside of the lid to the vertical position as shown in Figure 2. When the lever is in the vertical position, the cartridge protrudes by about 10 mm.
- 3 Gently pull the cartridge out of the lid as shown the Figure below.

Figure 2

Removing the 16-Pin Cartridge



# Cleaning procedure

## NOTE

After cleaning the electrode cartridge, do not use it for 2 days, to allow any water residuals to evaporate. Residual water will lead to bad performance of the instrument and eventually failed runs.

## Cleaning procedure:

- 1 While holding the assembly with the pins facing downward, gently but thoroughly brush the electrodes with a soft bristled toothbrush dipped in RNase Zap/RNase away for a few minutes, being careful not to get liquid into the openings on the electrode plate. The detergent ingredient in the RNase Zap will cause foam to form.
- 2 Let the cassette sit atop a beaker, with the pins faced downwards. Allow the electrode pins to be free from any contact.
- 3 After the foam has dissipated, brush the electrodes with RNase free water.
- 4 Follow by brushing the electrodes with ethanol.
- 5 Complete with RNase free water rinse.
- 6 Dry the electrodes with compressed air.

## WARNING

**Do not heat the electrode cartridge in an oven. The magnets inside may loose their magnetization. Thus the sensors can not detect the lid-closure.**

**7** As in step 2, place electrode assembly atop of beaker, allow it to dry for at least two hours.

**WARNING** Do not use the 2100 bioanalyzer for at least two days following the cleaning procedure.

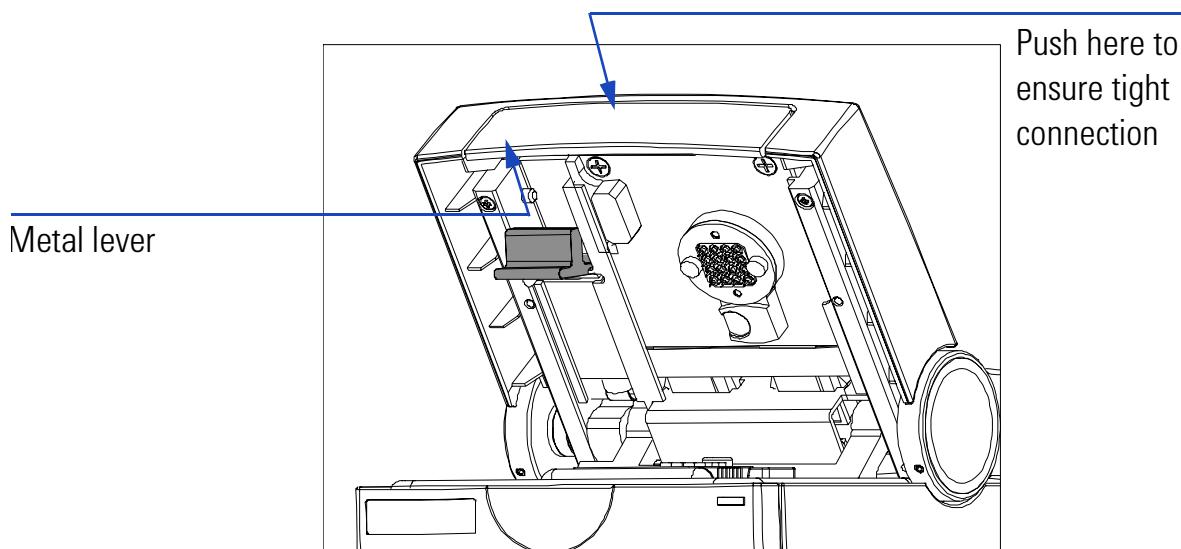
## Inserting the 16-Pin Cartridge

- 1 Slide the 16-pin cartridge in the lid as shown in the Figure 3.
- 2 Move the metal lever in the flat (closed) position.
- 3 Push the metal front of the 16-pin cartridge to ensure a tight connection to the 2100 bioanalyzer.

### NOTE

Make sure the 16-pin cartridge is connected tightly to the 2100 bioanalyzer.

Figure 3      Inserting the 16-Pin Cartridge



# Run the hardware diagnostics

Run the hardware diagnostics to ensure that the reader is functioning properly:

Perform the short circuit diagnostic test. See **Hardware Diagnostics—48** for details.

This test takes approximately three minutes and the software will walk you through the steps. When prompted, place an unused RNA or DNA chip into the reader (the chip can be used later for a regular run).

If the short circuit test fails, the assembly may still be wet. Take the assembly out of the instrument, dry it with compressed air and repeat the test.

**WARNING** Do not heat the electrode cartridge in an oven. The magnets inside may loose their magnetization. Thus the sensors can not detect the lid-closure.

**WARNING** Make sure that the electrode cartridge is completely dry before replacing it back into the electrode base. Even small amounts of liquid on the pin set can damage the high voltage power supply.



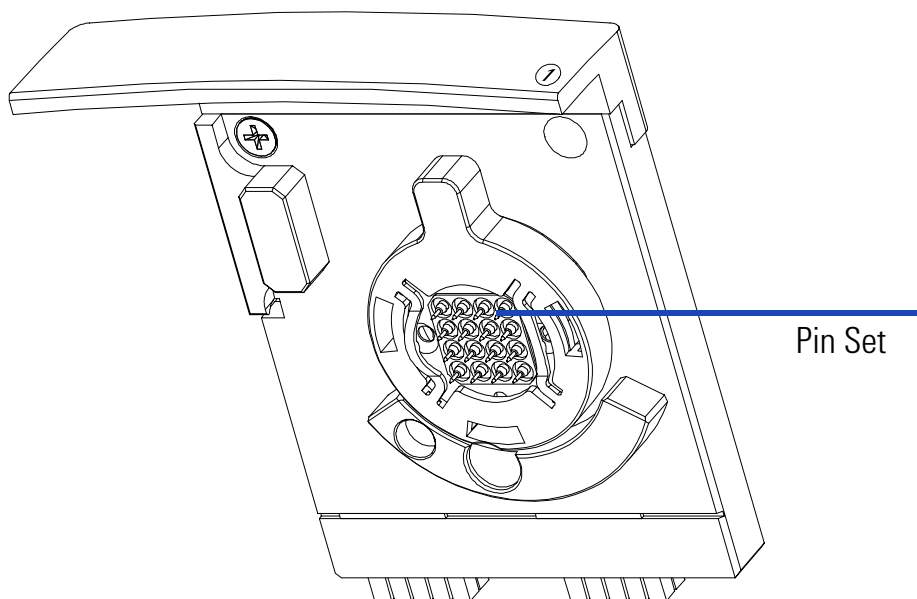
## Changing the 16-Pin Bajonet Cartridge

The 16-pin bayonet cartridge, see Figure 4, (reorder number 5065-4413) contains 16 electrodes. These are configured to fit in to the wells of a LabChip<sup>®</sup>.

The electrodes make contact with the liquid in the wells when the lid of the Agilent 2100 bioanalyzer is closed.

The cartridge, which includes the pin set, can be removed if the electrodes become contaminated or damaged.

Figure 4      16-Pin Cartridge and Pin Set of Cartridge



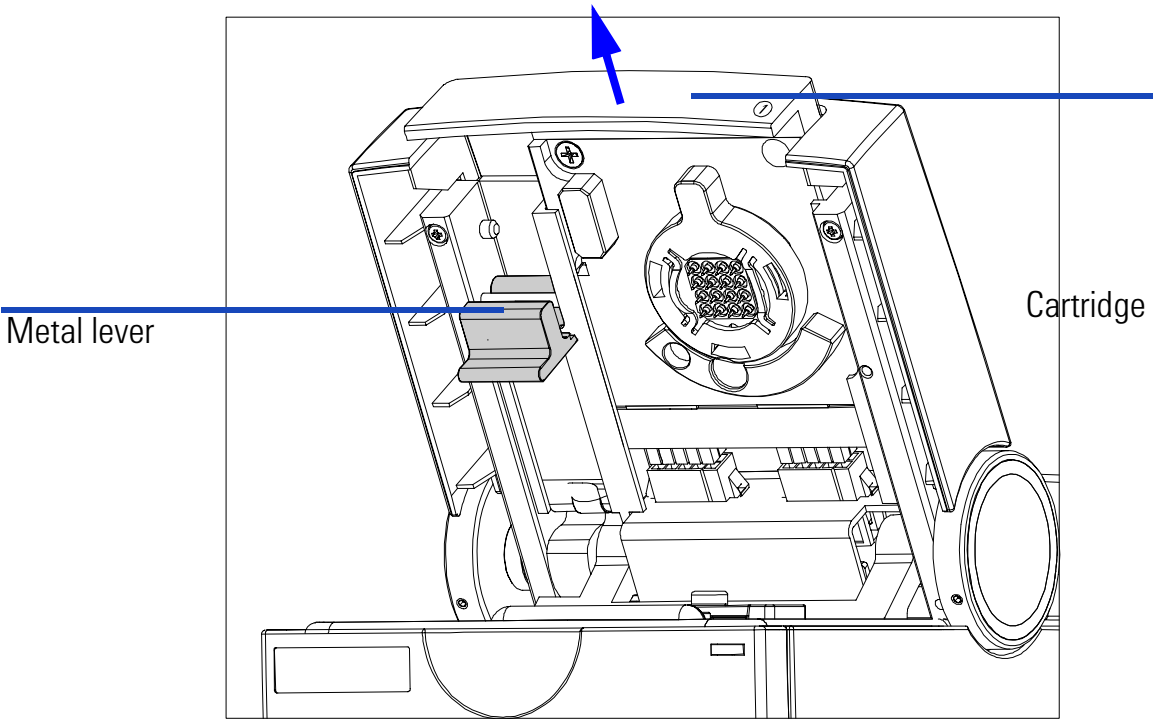
# Removing the 16-Pin Bajonet Cartridge

**WARNING** Do not touch the electrodes while the cartridge is in the 2100 bioanalyzer—this could cause damage to the electrodes and high voltage power supplies.

- 1 Turn off line power to the 2100 bioanalyzer.  
The line switch is located at the rear of the 2100 bioanalyzer.
- 2 Open the lid.
- 3 Pull the metal lever on the inside left of the lid to the vertical position as shown in Figure 2. When the lever is in the vertical position, the cartridge is released from the lid by about 10 mm.
- 4 Gently pull the cartridge out of the lid as shown in the Figure 5.

Figure 5

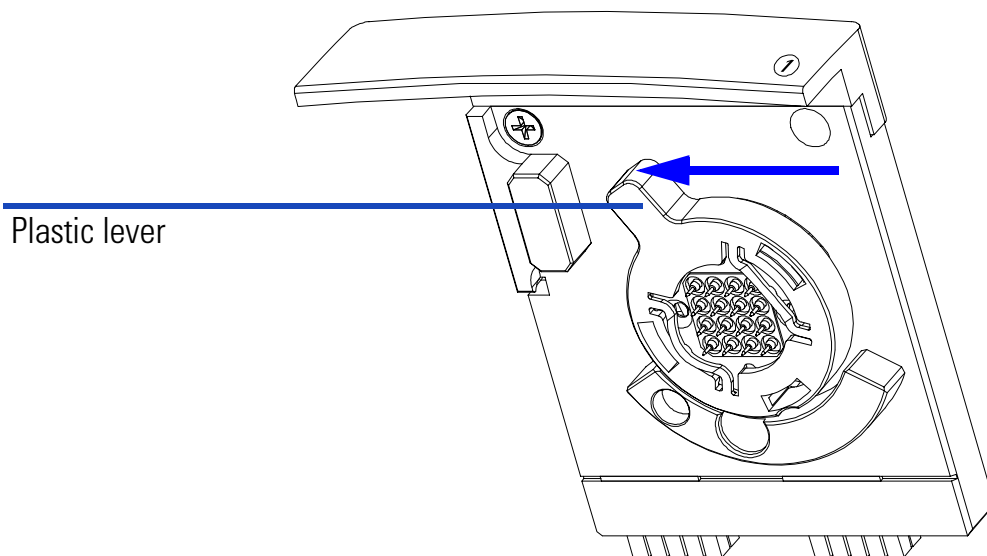
Removing the 16-Pin Cartridge



## Removing the Pin Set of the 16-Pin Cartridge

- 1 Remove the 16-pin cartridge as described above.
- 2 Open the bayonet socket of the pin set by turning the plastic lever to the left as described in Figure below.

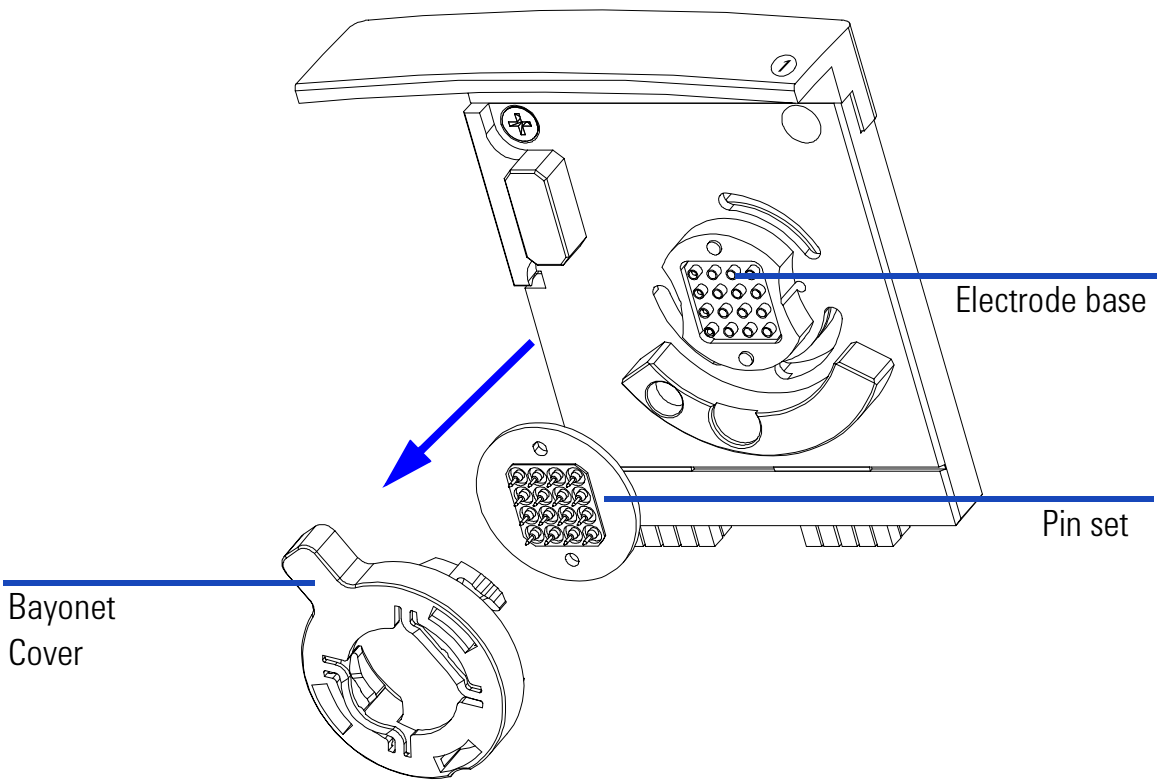
Figure 6      Opening the Bayonet Socket of the Pin Set



- 3 Remove the cover of the bayonet socket by gently pulling the plastic lever as shown in Figure 6. The pin set may stick to the electrode base. Remove it by carefully pulling it off. See Figure 7.

Figure 7

Releasing the Pin Set



**NOTE** For hints on how to clean the pin set, refer to [Cleaning the Pin Set of the 16-pin Cartridge—267](#)

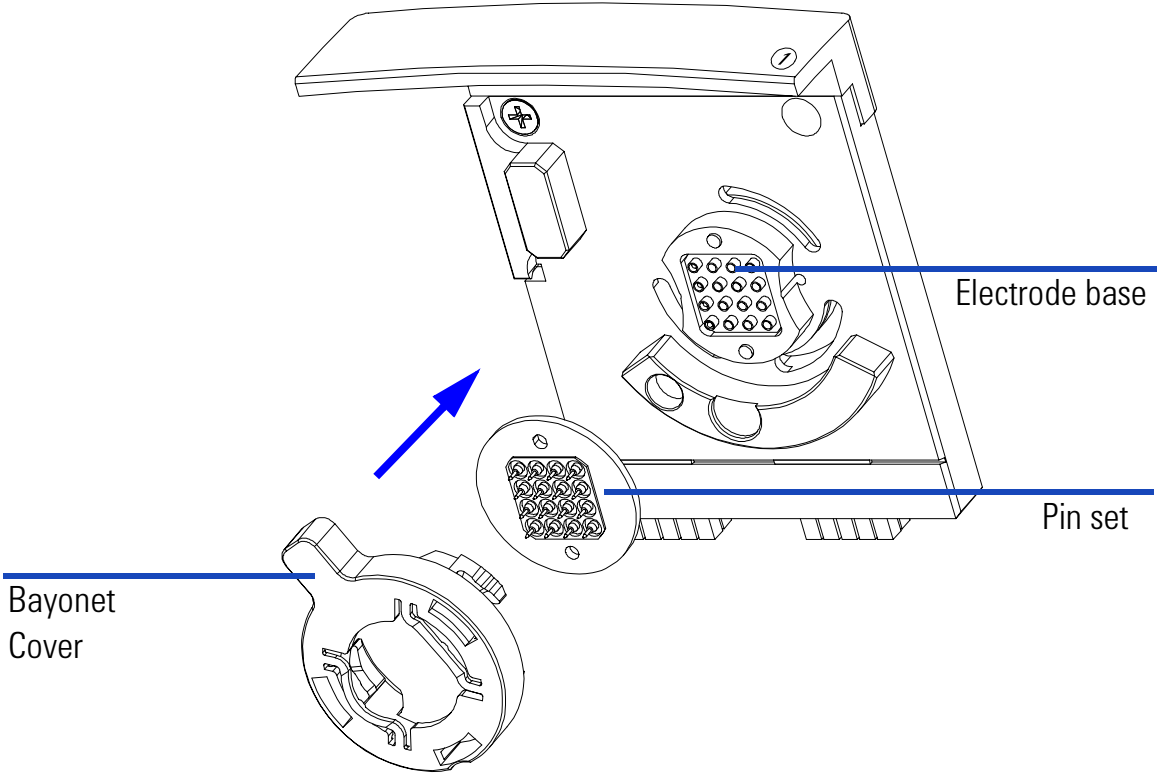
## Inserting the Pin Set of the 16-Pin Cartridge

**WARNING** Make sure that the pin set is completely dry before placing it back into the electrode base. Even small amounts of liquid on the pin set can damage the high voltage power supply.

- 1 Put the pin set on the cartridge base and the bayonet cover on the pin set. See Figure 8.

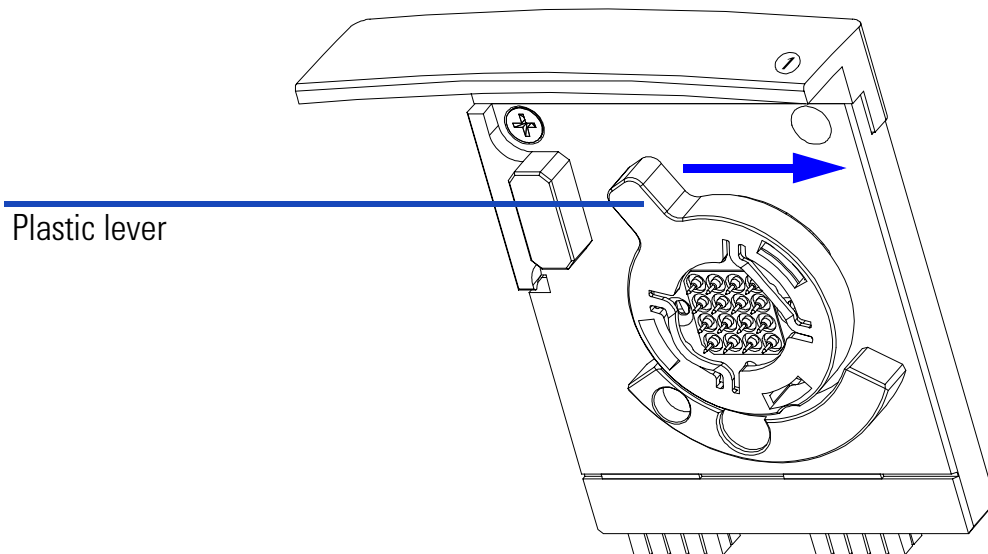
Figure 8

Inserting the Pin Set



- 2 Lock the pin set to the electrode base by pushing the plastic lever of the bayonet cover to the right as shown in Figure 9.

**Figure 9**      **Closing the Socket of the Pin Set**





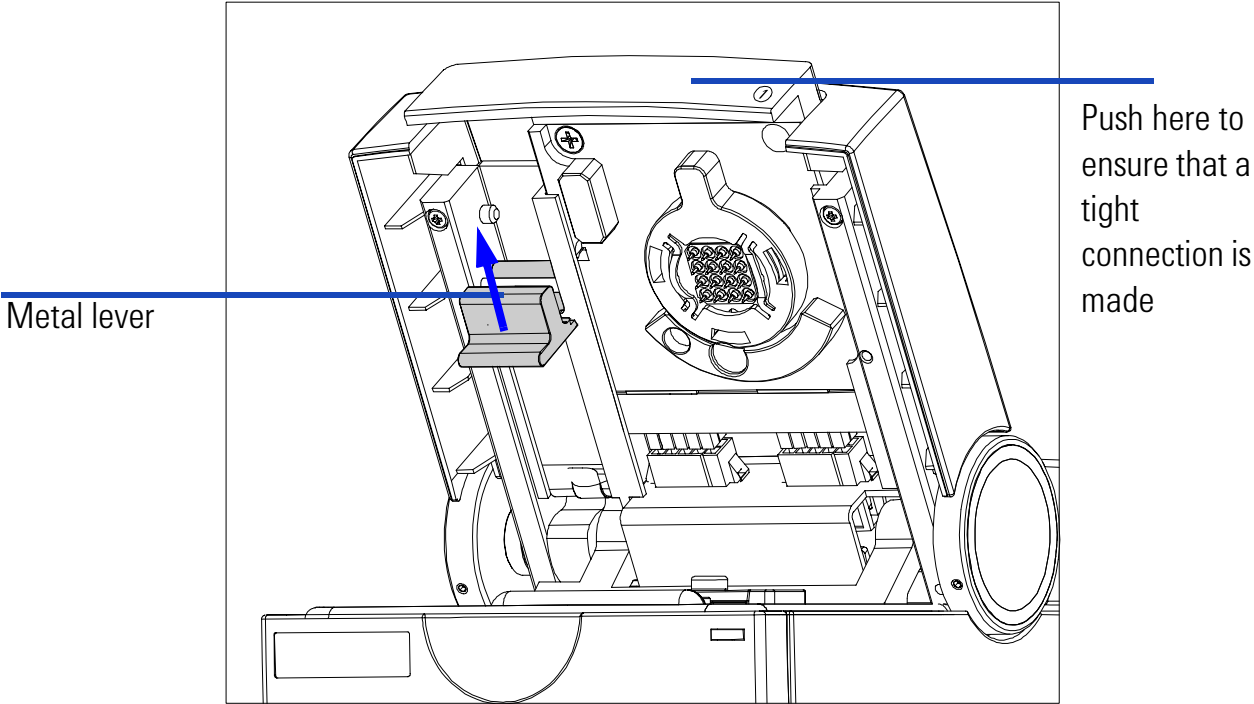
# Inserting the 16-Pin Cartridge

**WARNING** Make sure the pin set is be completely dry before putting in the 16-pin cartridge. Even small amounts of liquid on the pin set can damage the high voltage power supply of your instrument.

- 1 Slide the 16-pin cartridge into the bioanalyzer lid as shown in Figure on next page.
- 2 Move the metal lever in the flat (closed) position.
- 3 Push the metal front of the 16-pin cartridge to ensure a tight connection to the 2100 bioanalyzer.

Figure 10

Inserting the 16-Pin Cartridge



# Cleaning the Pin Set of the 16-pin Cartridge

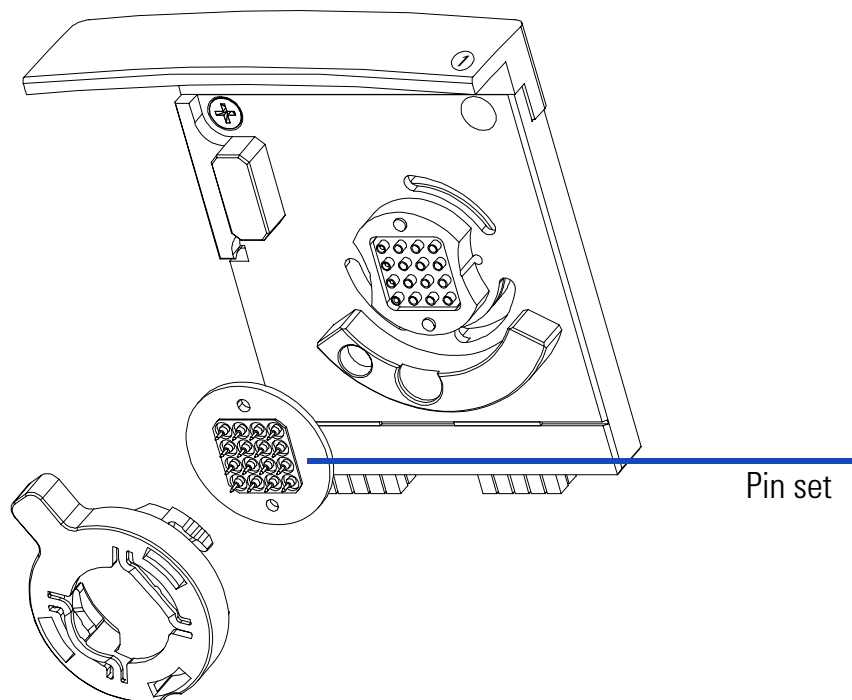
After removing the pin set from the 16-pin cartridge you can clean it by either using

- de-ionized water
- isopropanol

or

- RNase Zap

Figure 11 Pin Set



On a regular quarterly basis, or after contamination, gently clean the pin set with a lint-free surgical cotton swab damped in de-ionized water.

**WARNING** The pins of the pin set should not be bent or misaligned.  
Both will lead to poor quality results or pre-terminated assay runs.

In case of highly contaminated or dirty pins you may autoclave the pin set.

**NOTE** For autoclaving the pin set, follow your standard procedures for plastic material.

- 1 sonicate the pin set for 10 minutes.
- 2 gently clean the pin set with a soft tooth brush.

**WARNING** Make sure that the pin set is completely dry before replacing it back into the electrode base. Even small amounts of liquid on the pin set can damage the high voltage power supply.

# Run the hardware diagnostics

Run the hardware diagnostics to ensure that the reader is functioning properly:

Perform the short circuit diagnostic test. See **Hardware Diagnostics—48** for details.

This test takes approximately three minutes and the software will walk you through the steps. When prompted, place an unused RNA or DNA chip into the reader (the chip can be used later for a regular run).

If the short circuit test fails, the assembly may still be wet. Take the assembly out of the instrument, dry it with compressed air and repeat the test.

**WARNING** Do not heat the electrode cartridge in an oven. The magnets inside may loose their magnetization. Thus the sensors can not detect the lid-closure.

**WARNING** Make sure that the pin set is completely dry before replacing it back into the electrode base. Even small amounts of liquid on the pin set can damage the high voltage power supply.

## Lens Maintenance

Liquid spill may lower the light throughput of the focusing lens underneath the chip. To avoid reduced signal-to-noise ratios, or absorbing coatings on the lens, apply the following procedure on a quaterly basis or after liquid has been spilled on the lens.

- 1 Switch off the instrument. The line switch is located at the rear of the 2100 bioanalyzer.
- 2 Remove chip from the Agilent 2100 Bioanalyzer.
- 3 Dampen a lens tissue with reagent-grade isopropanol and gently swab the surface of the lens. Repeat several times with clean tissues and alcohol each time.

**WARNING** Do not drip liquid inside the instrument. Use special care to ensure safety.

- 4 Wait for alcohol to evaporate before use.

# Maintaining the Chip Priming Station

Perform the following two steps on a regular 3 months basis, after heavy use, or after scratching the tip of syringe or bending the syringe or the plunger or breaking the silicone gasket of the syringe.

Replace syringe and syringe adapter in order to ensure for proper sealing.  
Apply the seal test, in order to check if a replacement-gasket and syringe is needed.

## Replacing the Syringe Adapter (Part No. G2938-68716)

The Kit includes

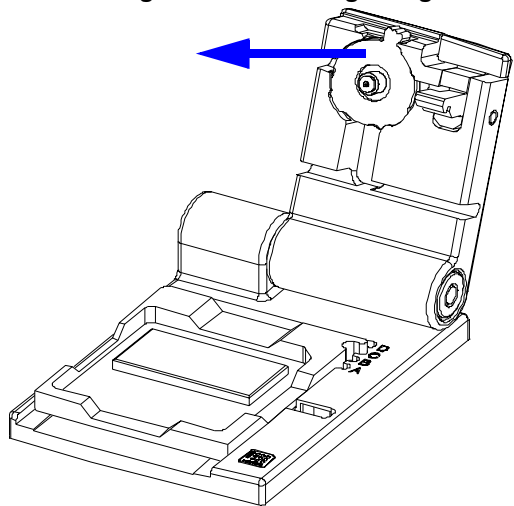
- 1 plastic adapter
- 1 mounting ring
- 10 silicon gaskets

### Replacement procedure:

- 1 Remove the syringe by gently pulling it out of the adapter.
- 2 Open the Chip Priming Station.
- 3 Move the ring holding the adapter in place to the left as shown in Figure 12. The ring will come off.

Figure 12

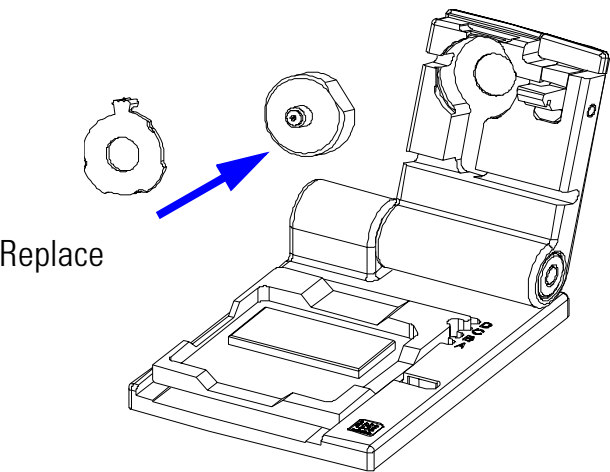
Releasing the Mounting Ring of the Syringe Adapter



4 Press the syringe adapter out of its mount, and replace it, as shown in Figure 13.

Figure 13

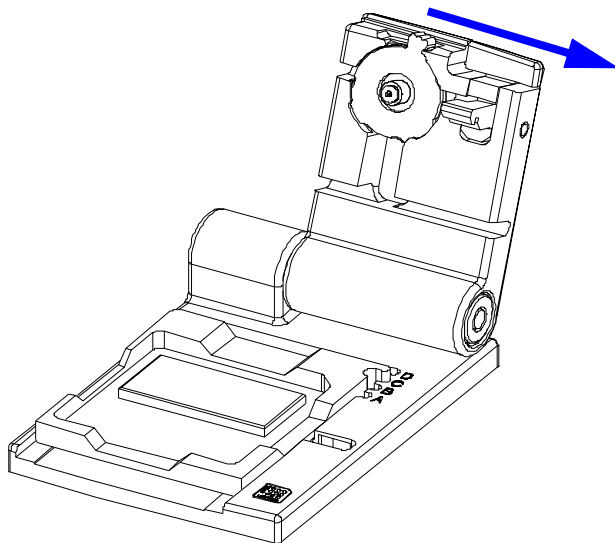
Replacing the Syringe Adapter (Part No. G2938-68716)





- 5** Put the mounting ring and the syringe adapter back in. Move the ring to the right in order to fix the syringe adapter as shown in Figure 14.

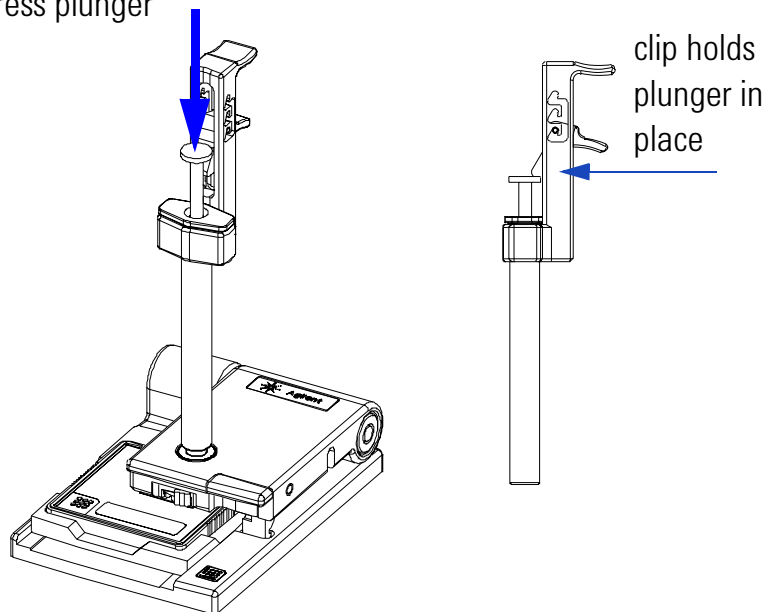
**Figure 14**      **Fixing the Syringe Adapter**



## Checking the Chip Priming Station for Good Seal — Seal Test

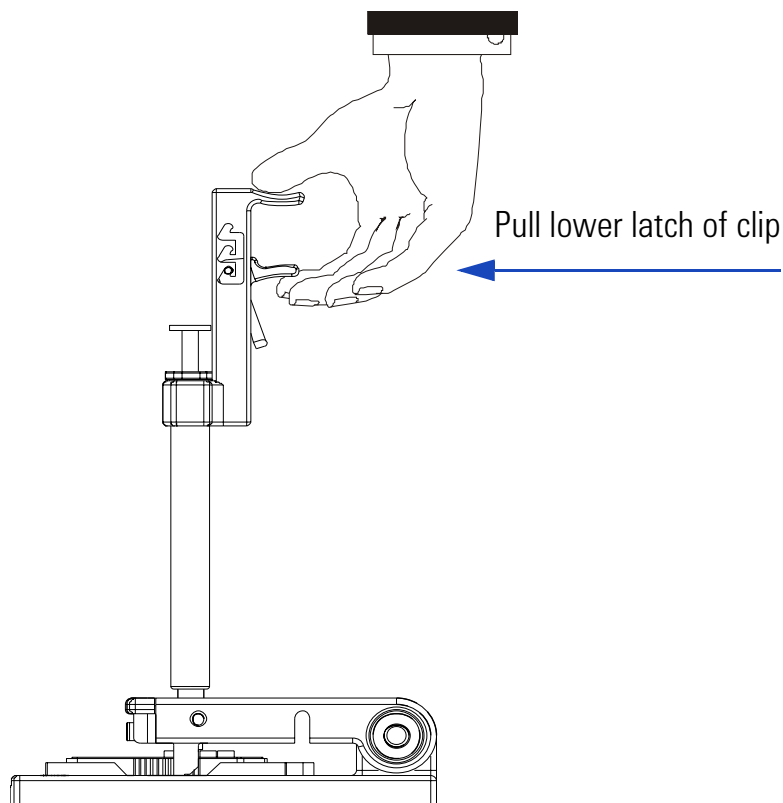
- 1 Make sure the syringe is tightly connected to the Chip Priming Station.
- 2 Pull the plunger of the syringe to the 1.0 ml position (plunger pulled back).
- 3 Place an empty chip in the Chip Priming Station.
- 4 Close the Chip Priming Station and make sure to lock it by pressing the cover. The lock of the latch will audibly click when it closes!
- 5 Press the plunger down until it is locked by the clip. This is shown in Figure 15.

Figure 15      Locking the Plunger of the Syringe with the Clip  
press plunger



- 6** Wait for 5 seconds and press the side of the clip to release the plunger as shown in Figure 16.

**Figure 16**                      **Releasing the Plunger of the Syringe**



- 7** Appropriate sealing is verified, if the plunger moves back up to the 0.3 ml mark within less than 1 second.

## NOTE

If the plunger doesn't move up to the 0.3ml mark within a second, the syringe-chip connection is probably not tight enough. Re-tighten the syringe and/or replace the syringe adapter and/or syringe to fix the problem.

### If Seal Test fails:

A failed seal test indicates the gasket must be replaced.

#### Replacement procedure:

- 1 Use a needle to pull out the silicon gasket.

## NOTE

Avoid scratching the plastic adapter when removing the silicon gasket.

- 2 Insert new gasket and gently push into place.
- 3 Apply the seal test.

Figure 17      Syringe Adapter with Gasket

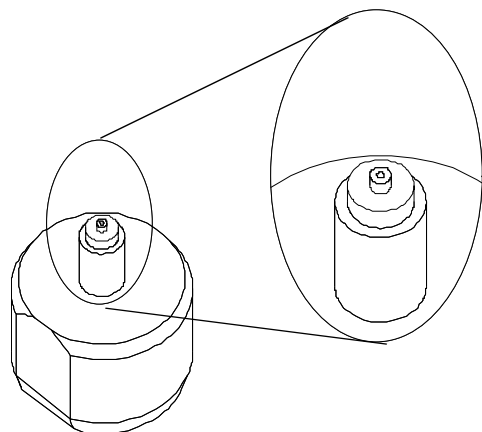
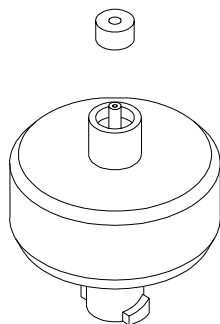


Figure 18      Disassembled Syringe Adapter



# Changing the Fuses of the Agilent 2100 Bioanalyzer

You need to change the fuses if the status indicator is off and the cooling fan is not running.

## Material Needed

Refer to for the type of fuses needed.

Specifications of Fuses

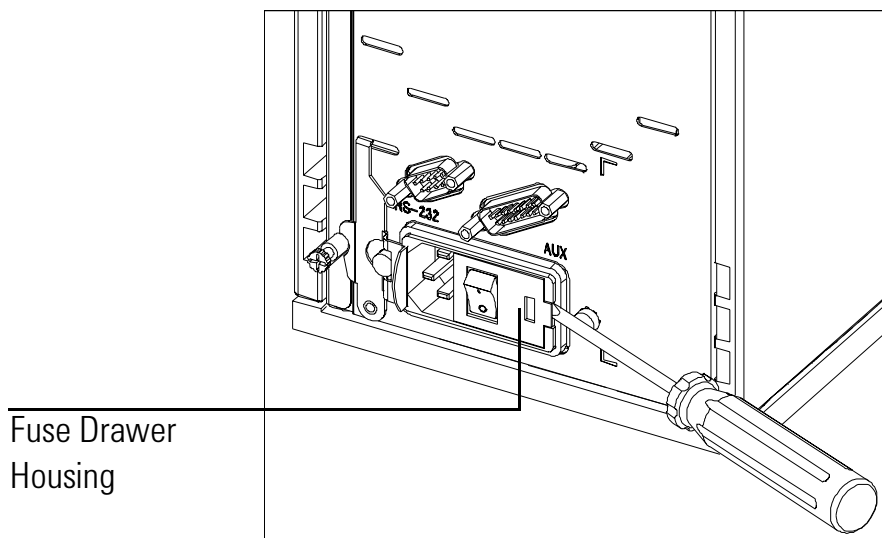
Description	Part Number	Number of Items
Fuse T 1A 250V	2110-0007	2

## Changing the Fuse

**WARNING** Disconnect the Agilent 2100 Bioanalyzer from line power before changing a fuse.

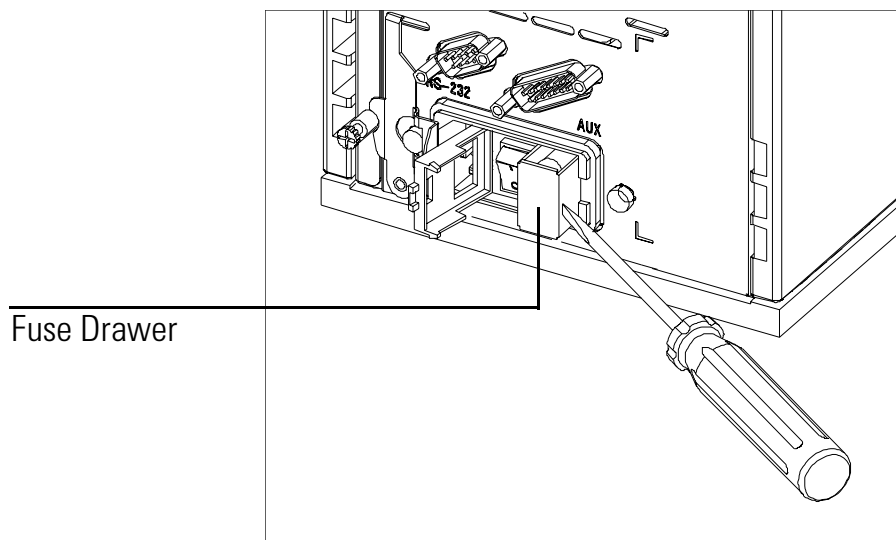
- 1 Disconnect the power cable from the power input socket at the rear of the Agilent 2100 Bioanalyzer.
- 2 To access the fuse drawer, gently lift the outer plastic housing of the power inlet socket using a screwdriver, see below.

Lifting the Housing of the Power Inlet



**3** Pull out the fuse drawer as shown in the next figure.

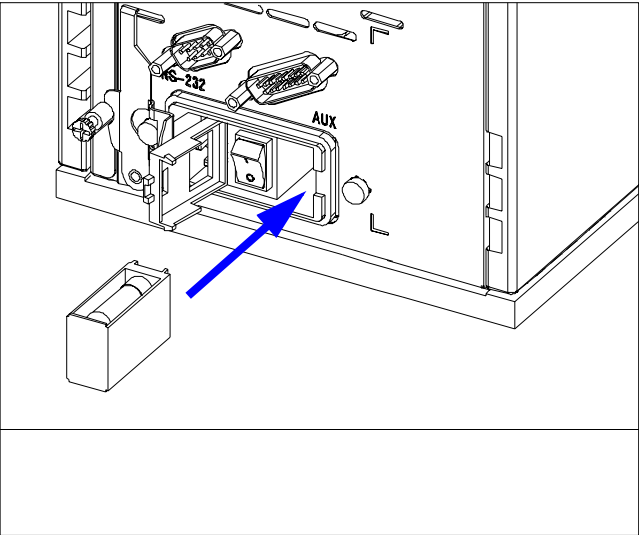
## Pulling out the Fuse Drawer



- 4** Check the fuses for conductivity and replace fuses if necessary.
- 5** Slide in the fuse drawer and push till it fits tightly as shown in the next figure.

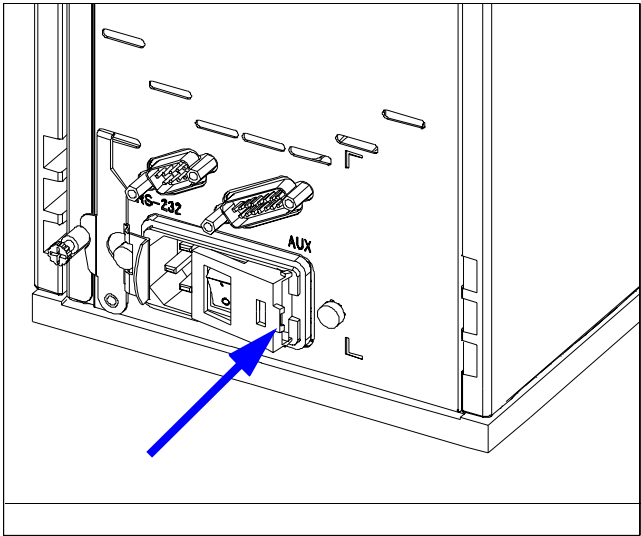


# Slide in the Fuse Drawer



**6** Slide in the fuse drawer and push till it fits tightly as shown in the figure.

# Close Fuse Drawer Housing



**7** Finally, close the fuse drawer housing and reconnect the instrument to the power line.

# Parts and Accessories

The following parts are available for the Agilent 2100 Bioanalyzer:

## **Bundles:**

For up-to-date details refer to:

<http://wadnts02.wad.hp.com/off/sc/pages/unsec/bundlist.htm>

Agilent 2100 Bioanalyzer Single-Instrument System G2940AA

VL400 PC, Instrument, accessories, printer, vortexer

Agilent 2100 Bioanalyzer Multi-Instrument System G2942AA

VL800 PC, instrument, accessories, printer, vortexer

Agilent 2100 Bioanalyzer Laptop System G2943AA

Omnibook 6000, instrument, accessories, printer, vortexer

**Hardware / Software**

Agilent 2100 Bioanalyzer G2938B

comprises 1 chip priming station, 1 testchip kit, serial cable, site&safety manual, setup poster

Agilent 2100 Bioanalyzer System Software G2941AA

Agilent 2100 Bioanalyzer Data Organizer G2945AA

## Reagent Kits and Reagents

RNA 6000 Nano Kit 5065-4476

Chips, reagents, Kit Guide, Syringe Box

DNA 500 Kit 5064-8284

Chips, reagents, Kit Guide, Syringe Box

DNA 1000 Kit 5065-4449

Chips, reagents, Kit Guide, Syringe Box

DNA 7500 Kit 5064-8230

Chips, reagents, Kit Guide, Syringe Box

DNA 12000 Kit 5064-8231

Chips, reagents, Kit Guide, Syringe Box

Protein 200 Plus Kit 5065-4480

Chips, reagents, Kit Guide, Syringe Box

RNA 6000 Nano Reagents 5065-4475

Cooled Reagents

DNA 500 Reagents 5065-4440

Cooled Reagents

DNA 1200 Reagents 5065-4438

Cooled Reagents

DNA 7500 Reagents 5065-4437

Cooled Reagents

Protein 200 Plus Reagents 5065-4482

Cooled Reagents

## **Accessories**

Vortex Mixer Adapter 5022-2190

for IKA vortexer

16-pin cartridge - bayonet 5065-4413

no extra electrode pin set; pin-set not re-orderable

Chip Priming Station 5065-4401

comprises 1 gasket kit, 1 adjustable clip

TestChip Kit G2938-68100

comprises 1 autofocus, 1 Electrode/Diode, 5 Leak Current Clips

## **Spare Parts**

RS 232 Cable G2938-81605

communication cable PC-instrument

Fuse 2110-0007

two fuses needed for G2938A

Gasket Kit G2938-68716

comprises 1 plastic adapter, 10 gaskets

Adjustable Clip 5042-1398

for use with luer lock syringe

Multiport Cable G2938-81610

for rocketport card



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